Unravelling the effects of multiple cross-ecosystem subsidies on food webs

A thesis submitted for the degree of

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by

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Dedicated to Victoria and Murray Greig

“All you have to do is make sentences…”

- Angus McIntosh

ABSTRACT

Adjacent ecosystems are connected by the exchange of resources across ecosystem boundaries, also known as cross-ecosystem subsidies, which can directly influence consumers and indirectly influence food-web interactions, such as bottom-up propagation of energy and top-down trophic cascades. The trophic level at which a subsidy enters a recipient ecosystem has the potential to alter consumer dynamics and mechanistic drivers of food-web interactions. Here I examined the effects of subsidy trophic level on bottom-up propagation of energy through food webs, top-down trophic cascades on primary producers, and reciprocal subsidies back to donor ecosystems. Results of a meta-analysis revealed results from past studies investigating effects of resource subsidies to primary producers, primary consumers, and predators on bottom-up energy propagation and top-down trophic cascades were inconsistent. The inconsistencies were likely due to differences in study duration or how subsidies were manipulated. To address some of the knowledge gaps revealed from the meta-analysis, I ran an 18-month pond mesocosm experiment, manipulating subsidies to primary consumers (terrestrial leaf subsidies), subsidies to predators (terrestrial insect subsidies), and presence of top predators (fish). Subsidy trophic level significantly altered food-web structure, with terrestrial insect subsidies increasing biomass of intermediate and top predators, while terrestrial leaf subsidies increased biomass of intermediate predators, increased isotopic niche widths of primary consumers, and altered community composition of invertebrates. However, although subsidies altered biomass and composition within trophic levels, subsidy frequency and strong top-down control had overriding influences on food-web interactions. Subsidy effects on trophic cascade strength varied through time and with subsidy trophic level; insect subsidies only increased trophic cascades early in the experiment, whereas leaf subsidy effects on trophic cascade strength increased with experiment duration. Interestingly, insect and leaf subsidies not only altered top-down interactions, but also influenced temporal and spatial variation of primary consumer and primary producer biomass. The input of subsidies to predators increased the total biomass and rate of aquatic insect emergence, compared to dampened emergence with subsidies to primary consumers, thereby creating sources and sinks of terrestrial energy. Overall, results suggest that altering the flow of energy subsidies entering at different trophic levels, not only

alters bottom-up and top-down interactions within the recipient food web, but can affect the extent to which exchanges of resources are reciprocal.

PREFACE

My thesis consists of four stand-alone papers intended for publication in peer-reviewed journals, followed by a general discussion chapter at the end. Therefore, there will be inevitable repetition amongst chapters, particularly in the introductory material and methods. The individual chapters consist of a meta-analysis and three data chapters based on results from one 18-month experiment.

Chapter One is a meta-analysis of previous work investigating influences of subsidies on bottom-up and top-down interactions within recipient ecosystems. Published work was analysed by subsidy trophic level (the trophic level at which a subsidy is incorporated into the recipient food web) to determine if subsidy trophic level drove differences in the effects of subsidies on trophic-cascades. Subsidy trophic level did not significantly alter trophic cascade strength, with highly variable results amongst studies investigating the subsidies of the same trophic level. This identified multiple knowledge gaps related to how subsidy trophic level alters bottom-up propagation of resources, top-down trophic cascades, and reciprocal exchange of subsidies between highly connected ecosystems, which were investigated in the rest of the thesis.

Chapter Two is the first of three chapters based on an 18-month manipulation of terrestrial insect and terrestrial leaf subsidies to freshwater food webs, as well as the presence of top fish predators. My aim was to investigate how the trophic level at which a subsidy entered recipient food webs affected food-web structure

Chapter Three describes how subsidising different trophic levels altered top-down trophic cascades. This chapter was based on biomass estimates of primary consumers and primary producers (benthic algae) collected on six sampling dates over the course of the experiment.

Chapter Four described how the experimental manipulation of subsidy trophic level affected emergence of aquatic insects. The results are based on emergence of freshwater invertebrates from experimental tanks over the course of 6 months, spanning spring to late summer/autumn.

Lastly, Chapter Five is a closing discussion reflecting on the knowledge gaps identified in the meta-analysis and how results from the experimental manipulation start to

fill in those gaps. The chapter ends with comments on the implications of my results in the face of increasing human modification of cross-ecosystem subsidies.

Chapters One to Four will be co-authored with my supervisors and some peers. With the thesis being based on an 18-month experiment that required upkeep every 2 weeks and sampling every two to three months, I received assistance from multiple people with field and with laboratory work. These helpers are listed and thanked in the acknowledgements. However, the majority of the work, including the ideas, data analysis, and writing, is my own. A complete reference list is at the end of the thesis, as opposed to at the end of each chapter. Also, several chapters include appendices, which will be included as supplementary material in published manuscripts.

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Chapter One: SUBSIDY TROPHIC LEVEL AFFECTS BOTTOM-UP AND TOP-DOWN FOOD WEB INTERACTIONS: A META-ANALYSIS

INTRODUCTION

The connection of food webs across landscapes, through the exchange of resource subsidies, has far-reaching influences on trophic interactions (O' Neill 2001, Richardson and Sato 2015, Soininen et al. 2015). The direct effect of subsidies on recipient consumer biomass and diversity can transfer to other trophic levels, resulting in an increase of predators at the tops of food webs (Marczak et al. 2007). Combined with the increase in consumers, subsidies can also disconnect feedbacks between consumers and their resources, potentially increasing destabilising forces due to top-down food-web interactions, such as trophic cascades (Huxel and McCann 1998, Leroux and Loreau 2008). However, not all subsidies perform the same function within recipient food webs (Marcarelli et al. 2011, Hagen et al. 2012a), and the trophic level at which subsidies enter food webs may alter trophic cascade strength (Leroux and Loreau 2008).

The trophic level at which a subsidy enters is likely to be important because additional resources provided by cross-ecosystem subsidies can directly increase consumer biomass (Polis et al. 1997) and diversity (Anderson and Wait 2001, Dugan et al. 2003) either at the top or base of food webs. The lower the subsidy trophic level (i.e. subsidies to primary producers or consumers), the more likely the subsidy is to affect recipients at low trophic levels (Bastow et al. 2002), whereas high trophic level subsidies maybe more likely to have a strong effect on predators (Greenwood and McIntosh 2008, Marczak and Richardson 2008). However, increases in resources at any trophic level can propagate up food webs to affect predators and alter whole food-web dynamics (Polis and Hurd 1995, Carpenter et al. 2005, Spiller et al. 2010). Thus, propagation of the effects of subsidies within recipient ecosystems is potentially highly dependent on the subsidy trophic level.

Glossary

Primary producer: Organism that produces biomass from abiotic nutrients and sunlight and has produced that biomass within the focal ecosystem.

Primary consumer: Organism that feeds on primary producers (a.k.a herbivores).

Intermediate predator: Organism that feeds on primary consumers but is not the highest trophic level.

Predator: Organism at the highest trophic level of the food web that feeds on intermediate predators and primary consumers.

Subsidy: Material or organisms (abiotic or biotic) that are produced outside of the recipient ecosystem.

Recipient ecosystem: Ecosystem in which the subsidy is being received.

Donor ecosystem: Ecosystem in which the subsidy was produced.

Subsidy to primary producers: Nutrients that have been produced in a donor ecosystem and transfered to a recipient ecosystem. They are taken into the food web by primary producers. Usually a form of nitrogen (N) or phosphorous (P). Also categorised as **Abiotic subsidies.**

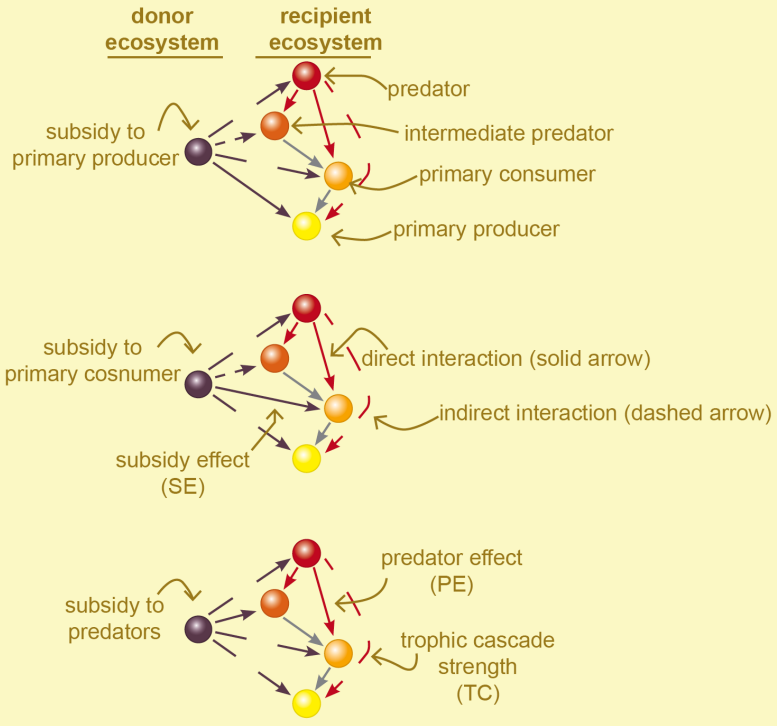
Subsidy to primary consumers: Primary producer biomass that was produced within a donor ecosystem and transported to a recipient ecosystem. They are incorporated into the recipient food web through consumption by primary consumers. E.g. Terrestrial leaves in stream or seaweed on beaches. Also categorised as **Biotic subsidies.**

Subsidy to predators: Primary consumer biomass that was produced within a donor ecosystem and transported to a recipient ecosystem. They are incorporated into the recipient food web through consumption by predators. E.g. Aquatic insects consumed by spiders or terrestrial arthropods consumed by fish. Also categorised as **Biotic subsidies.**

Top-down predator effect (PE): Direct and indirect effects that predators have on lower trophic levels (intermediate predators, primary consumers, primary producers). See methods for details on formula.

Trophic cascade strength (TC): Top-down indirect predator effects on primary producers.

Bottom-up subsidy effect (SE): Direct and indirect effects that subsidies have on different trophic levels (predators, intermediate predators, primary consumers, primary producers) within recpient food webs. See methods for details on formula.



Bottom-up subsidy effects that increase predator abundance can strengthen top-down predator effects to *in situ* consumers (Sabo and Power 2002), releasing primary producers from consumption and creating trophic cascades (Nakano et al. 1999, Huxel et al. 2002). Theoretically, trophic cascade strength is likely to increase as subsidy input increases (Huxel and McCann 1998, Huxel et al. 2002, Leroux and Loreau 2008). However, empirical studies report highly variable relationships between subsidy input and trophic cascade strength (Nakano et al. 1999, Henschel et al. 2001, Piovia-Scott et al. 2011).

Differences in the trophic level at which subsidies enter food webs could explain this observed variability within empirical evidence regarding the influence of subsidies on trophic cascade strength. Polis and Hurd (1995) found that subsidies to primary consumers increased primary consumer abundance, which numerically propagated up to inflate predator density above what could be supported by *in situ* productivity, thereby creating a top-down cascade to plants. On the other hand, Nakano et al. (1999) found that increasing subsidies to predators caused behavioural prey-switching by predators from *in situ* primary consumers to terrestrial insect subsidies, increasing primary consumer densities. Increases in primary consumers then cascaded down to decrease primary producer biomass, thus decreasing trophic cascade strength (Nakano et al. 1999). Thus the differential effects of subsidies to primary consumers versus subsidies to primary producers may explain the variety of effects of subsidies on trophic cascades. Another thing to consider is the timescales at which the above two processes (i.e. behavioural prey-switching versus numerical propagation) involved in the influence of subsidies to different trophic levels take place.

Mechanistically, subsidies affect consumers and subsequent indirect interactions (such as apparent competition with *in situ* resources and trophic cascades) in two ways: short-term behavioural prey-switching or longer-term numerical increases in abundance/biomass (Baxter et al. 2005). Short-term behavioural prey-switching of consumers to subsidies, through apparent competition, relieves *in situ* resources from consumption increasing their abundance/biomass, as seen by Nakano et al. (1999). However, models by Leroux and Loreau (2008), as well as a review of empirical work by Polis et al. (1997), predict that over multiple generations, the increased subsidy resources will lead to numerical increases in consumers that propagate up food webs, supporting high numbers of predators. Inflated predator numbers could then exert strong top-down effects, increasing trophic cascades (Polis et al. 1997, Leroux and Loreau 2008). Therefore, study duration could potentially have a strong influence on whether subsidies increase trophic cascades, due to short-term versus longer-term processes.

The increasing number of published experimental manipulations of subsidies in recent years enables quantitative meta-analysis to disentangle mechanisms behind different responses among studies. I investigated three main questions. Firstly, I tested how subsidy trophic level affected the bottom-up and top-down transfer of energy through food webs. I was interested in whether subsidies that entered at low trophic levels had less influence on predators than subsidies that entered at higher trophic levels. I hypothesised that direct bottom-up effects of subsidies on consumers would be stronger without predators and that the direct effects to consumers would be stronger than indirect effects on other trophic levels. Secondly, I investigated whether the bottom-up propagation of subsidies to predators translated to stronger top-down forces, resulting in increased trophic cascade strength. I predicted that subsidies to predators would have a larger influence on trophic cascade strength than other subsidies, because they are direct resources to the predators that control the trophic cascades. Lastly, I was interested if study duration altered the magnitude of subsidy effects on trophic cascade strength. I predicted short, behaviourally-driven, trophic cascades would to be stronger than longer-term, numerically-driven, cascades.

METHODS

My meta-analysis used primary, empirical research found using a Web of Science search with the keywords: "trophic cascade subsid*", "trophic cascade cross ecosystem", and "top-down subsid*" as well as searches through studies included in recent meta-analyses on similar topics (Shurin et al. 2002, Marczak et al. 2007, Yang et al. 2010, Marcarelli et al. 2011, Bartels et al. 2012, Hagen et al. 2012b). Search criteria for studies included: 1) a manipulation of a subsidy or a naturally occurring gradient of subsidies; 2) either a manipulation or use of naturally occurring predator presence/absence; and 3) a quantitative measure of primary producers (biomass, density, etc) (Shurin et al. 2002, Marczak et al. 2007). Studies were not excluded for subjective quality issues (replication, poor experimental set-up, etc.), but were excluded if one or more of the above criteria were not met.

When results were reported in a time series, each sampling date was included as a separate data set within the meta-analysis. For studies with multiple predator and/or subsidy treatments, each treatment level combined with the control was considered a separate data set. If predator or subsidy treatments were fully crossed with other treatments (e.g. temperature), the data from other treatments were also considered as separate data sets. Papers were not included if the predator was also considered the source of subsidy, because predator and subsidy were not independent treatments, and therefore there was not a full

predator x subsidy cross (e.g. Kurle et al. 2008). When multiple primary producer, primary consumer, intermediate predators, or predator response variables were reported, each response was treated as a separate data set in the analysis. In instances when one measure was a subset of another (e.g. total primary consumer density and density of a particular functional group), then the more specific measurement was used (e.g. the particular functional group). Because subsidies are one of the treatments, only studies or data were included that had primary producers and herbivores as the response variables, so as to not confuse the response variable. Thus, I did not include detrital trophic cascades (top-down influence of predators on detrital breakdown), for example.

For each study, bottom-up subsidy effect sizes (SE) for each separate trophic level reported within a food web were calculated by using the natural log-ratio:

$$SE = \ln(S_1/S_0)$$

where S_1 was the trophic level response for treatments with high levels of subsidies and S_0 was the trophic level response for treatments with low levels of subsidies (or absence of subsidies) (Marczak et al. 2007). The subsidy effect size was calculated for low and high predator treatments separately. Top-down predator effect sizes (PE) for each separate trophic level reported within a food web were also quantified with the natural log-ratio:

$$PE = \ln(P_1/P_0)$$

where P_1 was the trophic level response for treatments with high levels of predators and P_0 was the trophic level response for treatments with low levels of predators (or absence of predators) (Shurin et al. 2002). The predator effect size was calculated for low and high subsidy treatments separately. The predator effect size on primary producers will hereafter be referred to as trophic cascade strength (TC). Log-ratios were used because they have clear biological meaning, sampling distribution is approximately normal, and showed the least statistical bias in a comparison of several effect size metrics (Hedges et al. 1999). Although confidence associated with log ratios can vary among studies, I did not weight effect size ratios because it would have reduced my sample size and may introduce bias (Gurevitch and Hedges 1999, Hedges et al. 1999). Data that were presented as log-transformed values were untransformed to calculate SE and PE.

The size of change in trophic cascade strength between low and high subsidy levels was calculated as:

$$|\Delta TC| = | PE_1 - PE_0 |$$

where PE_1 was the predator effect size on primary producers for high subsidy levels and PE_0 was the predator effect size on primary producers for low subsidy levels.

Table 1.1: An overview of the data sets obtained from 17 studies for the meta-analysis of the effects of subsidy trophic level on bottom-up and top-down interactions, and categorised by study system, subsidy type, and response trophic level. Subsidy trophic level is listed as the trophic level at which the subsidy was consumed and response trophic level is the trophic level within recipient ecosystems for which I could calculate subsidy and predator effect sizes.

Habitat	Subsidy trophic level	Response trophic level	Study*	data sets
	subsidy to:			
marine	primary producers	primary producer	8	4
lake	primary producers	primary producer	7, 14, 16	21
		primary consumer	7, 14, 16	32
	primary consumers	primary producer	4, 10	8
		primary consumer	4, 10	31
		intermediate predator	10	4
stream	primary producers	primary producer	3, 5, 6, 13	11
		primary consumer	3, 5, 6, 13	39
		predator	3, 6	20
	primary consumers	primary producer	12	1
		primary consumer	12	48
		intermediate predator	12	16
	predators	primary producer	1, 2, 11	4
		primary consumer	1, 2, 11	4
		intermediate predator	1, 2	3
		predator	1, 2	2
terrestrial	primary producers	primary producer	17	1
	primary consumers	primary producer	15	3
		primary consumer	15	1
		intermediate predator	15	1
		predator	15	1
	predators	primary producer	9	1
		primary consumer	9	8
		predator	9	10
total				**274**

*Studies 1-17 listed in Appendix 1.1

Statistical Analyses

A total of 275 data sets from 17 papers, from multiple habitats, subsidy types, and trophic levels (Table 1.1) were used in the meta-analysis. To test bottom-up subsidy effects on food webs with low and high predators, I ran linear models with interactions between predator level (low, high), subsidy type (nutrient, primary producer, primary consumer), and food-web trophic level (primary producer, primary consumer, intermediate predator,

predator) as the independent variables, and subsidy effect size as the dependent variable. To test top-down predator effects on food webs with low and high subsidies, I ran linear models with interactions between subsidy level (low, high), subsidy type (nutrient, primary producer, primary consumer) and food-web trophic level (primary producer, primary consumer, intermediate predator, predator) as the independent variables and predator effect size as the dependent variable. Type III sums of squares were used in all linear models to account for unbalanced treatments.

To investigate the specific effect of variations in subsidy amount on trophic cascade strength (top-down predator effect on primary producers), I ran a linear model with subsidy amount (low, high) as the independent variable and predator effect size on primary producers (PE) as the dependant variable. I also ran a model including independent variables, subsidy trophic level (subsidies to primary producers, primary consumers, and predators), subsidy amount (low, high), and their interactions with primary producer PE as the dependent variable.

Correlation between |ΔTC| and study duration was analysed using a linear model with the independent variable, study duration (days), and |ΔTC| as the dependent variable. Study duration was $\log_e$-transformed to meet assumptions.

Trophic cascade strength (TC) of low subsidy amount treatments was used to predict the direction of change (increase or decrease) in TC between low and high subsidy amounts. I used a generalised linear model (GLM) with binomial distribution (chi square test) to test the effects of the independent variable, TC of low subsidy treatments, with direction of change (increase or decrease) in TC from low to high subsidies as the dependant variable. To plot the GLM relationship, the "predict" function in R (R Core Team 2014) was used to estimate y-values based on the GLM model parameters.

The |ΔTC| was analysed using a linear model with independent variables, TC of low subsidy treatment, subsidy trophic level (subsidies to primary producer, primary consumers, and predators), and their interactions, with |ΔTC| as the dependent variable. To further investigate whether biotic (subsidies to primary consumer and predators) versus abiotic (subsidies to producers) subsidies influences on |ΔTC|, subsidy trophic level was re-categorized by combining primary producers and primary consumers into one factor. I used a linear model with the interaction between low subsidy trophic cascade strength and subsidy type (abiotic, biotic) as the independent variables and size of change in trophic cascade strength as the dependent variable. All statistical analyses were run using R (R Core Team 2014).

Table 1.2 Results for meta-analysis on subsidy trophic level altering bottom-up and top-down food-web interactions for response variables a) subsidy effect size (SE), b) predator effect size (PE), c) trophic cascade strength (TC), d&f) |ΔTC| which is the difference in trophic cascade strength between low and high subsidy amounts, and e) direction of change in trophic cascade strength from low to high subsidy amounts. Predictor variables are italicised, with SE trophic level indicating a change in subsidy effect between different trophic levels within a food web, and PE trophic level indicating a change in predators effect on different trophic levels within a food web. ANOVA = analysis of variance, LM = linear model, binomial GLM = binomial generalized linear model. Probability values (P) that are bold are significant (P<0.05) and F values presented as $F_{(\text{degrees of freedom variable, error})}$.

Response variable	**analysis**	**F**	**P**
a) Subsidy effect size (SE)	ANOVA		
SE trophic level		$9.32_{(3,402)}$	**<0.001**
predator amount		$1.44_{(1,402)}$	0.231
subsidy trophic level		$6.15_{(2,402)}$	**0.002**
SE trophic level x predator amount		$0.97_{(3,402)}$	0.404
SE trophic level x subsidy trophic level		$3.76_{(3,402)}$	**0.011**
predator amount x subsidy trophic level		$1.41_{(2,402)}$	0.245
SE trophic level x predator amount x subsidy trophic level		$0.09_{(3,402)}$	0.963
b) Predator effect size (PE)	ANOVA		
PE trophic level		$5.64_{(2,407)}$	**<0.001**
subsidy amount		$1.72_{(1,407)}$	0.189
subsidy trophic level		$0.88_{(2,407)}$	0.411
PE trophic level x subsidy amount		$1.01_{(2,407)}$	0.362
PE trophic level x subsidy trophic level		$0.86_{(3,407)}$	0.458
subsidy amount x subsidy trophic level		$2.48_{(3,407)}$	0.084
PE trophic level x subsidy amount x subsidy trophic level		$0.15_{(3,407)}$	0.926
c) Trophic cascade strength	ANOVA		
subsidy amount		$0.46_{(1,104)}$	0.419
subsidy trophic level		$1.47_{(2,104)}$	0.234
subsidy amount x subsidy trophic level		$1.03_{(2,104)}$	0.357
d) |ΔTC|	LM		
*study duration (days)**		$1.30_{(1,51)}$	0.259
abiotic/biotic subsidy type		$0.54_{(1,51)}$	0.463
study duration x abiotic/biotic subsidy type		$4.66_{(1,51)}$	**0.035**
study duration (days) - abiotic subsidies*		$0.08_{(1,36)}$	0.771
study duration (days) - biotic subsidies*		$6.39_{(1,15)}$	**0.023**
e) Direction of change in trophic cascade strength	bionomial GLM		
TC of low subsidy treatment	chi square test	-	**0.018**
f) |ΔTC|	LM		
TC of low subsidy treatment		$11.78_{(1,51)}$	**0.001**
abiotic/biotic subsidy type		$1.20_{(1,51)}$	0.278
TC of low subsidy treatment x abiotic/biotic subsidy type		$5.59_{(1,51)}$	**0.021**
TC of low subsidy treatment - abiotic subsidies		$1.46_{(1,36)}$	0.233
TC of low subsidy treatment - biotic subsidies		$41.72_{(1,15)}$	**<0.001**

* $\log_e$ transformed data

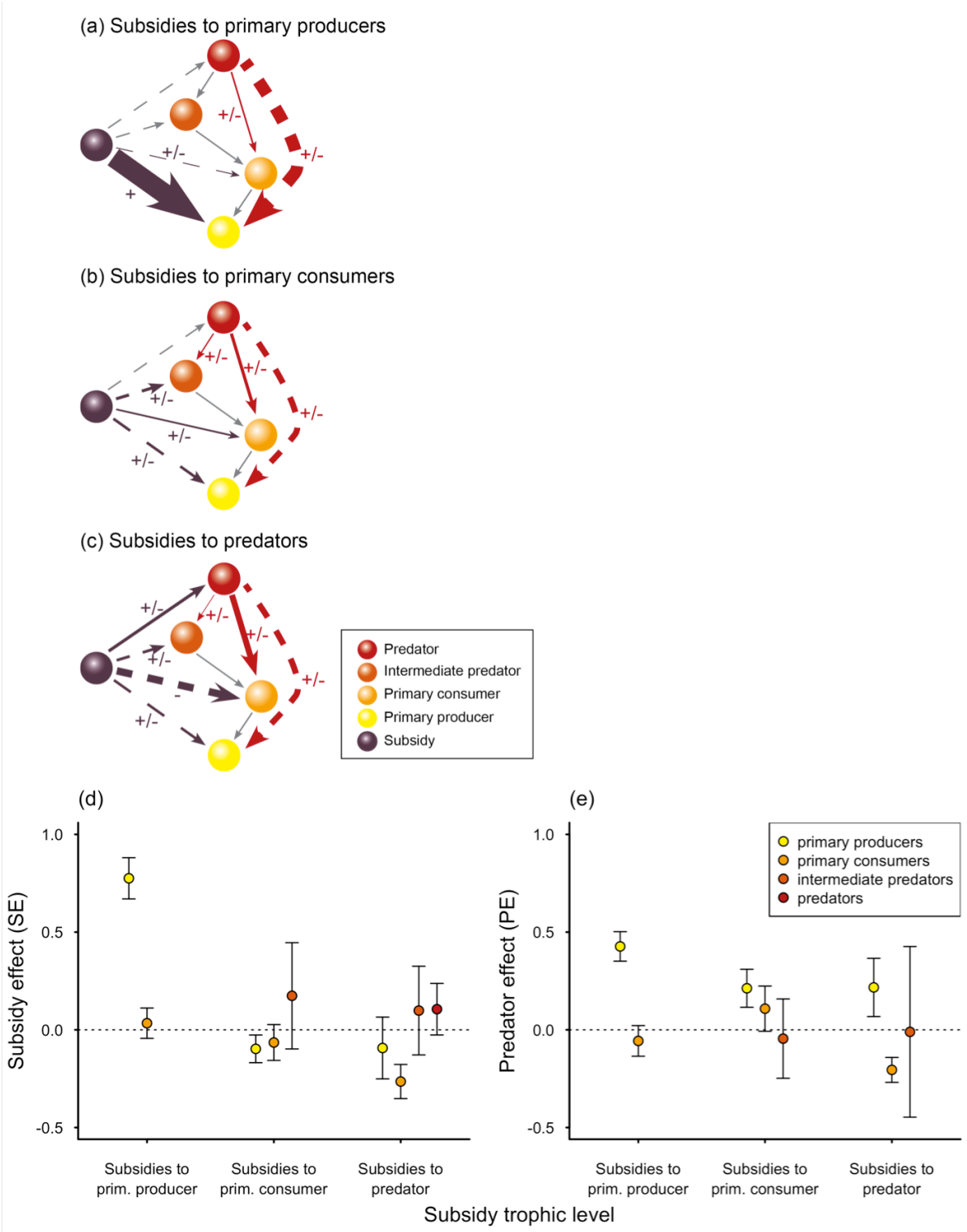


Figure 1.1: Meta-analysis results for the influence of trophic level on bottom-up subsidy effects and top-down predator effects. Mean strength of top-down predator effects (red arrows) and bottom-up subsidy effects (purple arrows) on food webs for (a) subsidies to primary producers, (b) subsidies to primary consumers, and (c) subsidies to predators and mean +/- 1 standard error of the mean for (d) subsidy effect and (e) predator effect. The size of the arrow is proportional to the effect size and (+) indicate a positive effect and (-) a negative effect and (+/-) effects that spanned positive and negative. Solid arrows represent direct interactions, dashed arrows represent indirect interactions, grey arrows represent links that were not tested within the meta-analysis.

RESULTS

I analysed 274 data sets from 17 papers, which included marine, lake, stream, and terrestrial habitats (Table 1.1; Appendix 1.1). All habitats, except for marine, included studies that manipulated subsidies to primary producers, primary consumers, and predators. However, all data sets for certain habitat and subsidy trophic level combinations came from one published study, including all terrestrial habitat and subsidy trophic level combinations (Table 1.1; Appendix 1.1). Therefore, there is a degree of bias within the results, because a majority of data sets were a combination of freshwater (stream and lake) habitats where subsidies to primary producers were manipulated (nutrients; Table 1.1).

Out of the 17 published papers, 15 reported on subsidies altering trophic cascade strength. Of those, seven reported increased trophic cascades with increasing subsidies, three reported decreased trophic cascades with increased subsidies, and five reported no change in trophic cascade strength.

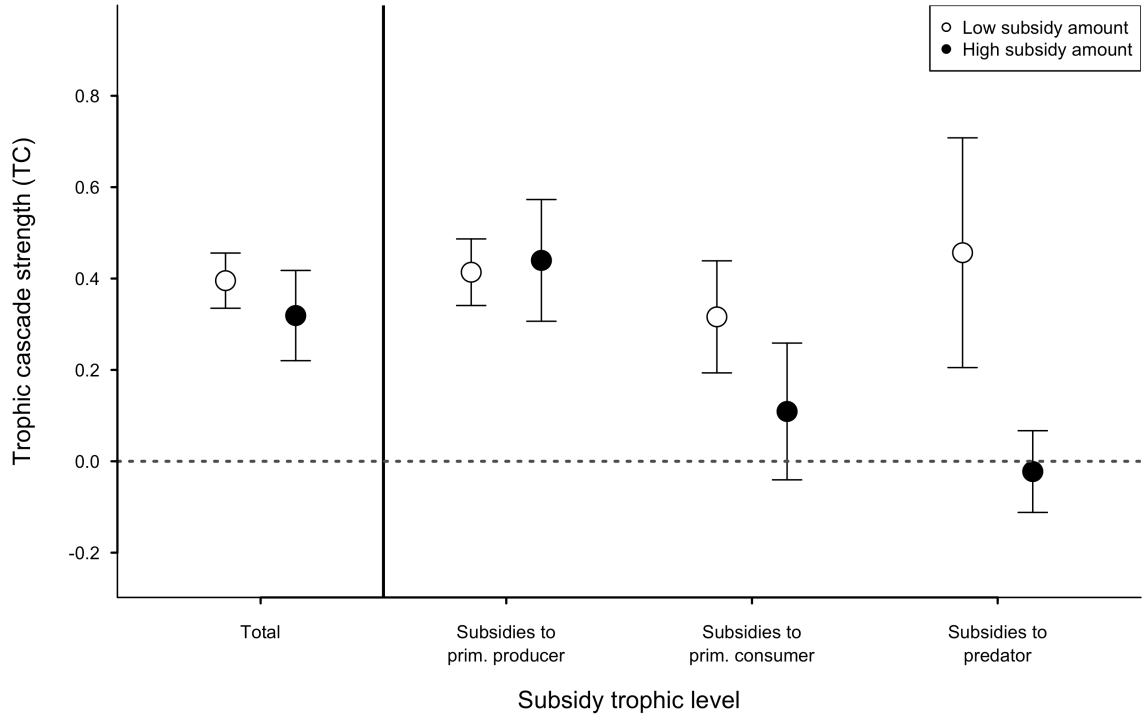


Figure 1.2: Mean of top-down predator effecs on primary producers, i.e. trophic cascade strength (TC) between low (open points) and high subsidy (closed points) amounts for subsidies entering at different trophic levels (Subsidy trophic level). Error bars are +/- 1 standard error of the mean.

Bottom-up and top-down food-web interactions

Subsidy effects (SE) on recipient food-web trophic levels (SE trophic level) were significantly altered by the trophic level at which the subsidy entered the system (SE trophic level x subsidy trophic level interaction, p=0.011; Table 1.2a; Figure 1.1). This was made apparent by strong direct increases in producers from subsidies to producers (Figure 1.1a&d) and strong negative indirect decreases in primary consumers with subsidies to predators (Figure 1.1c&d). Interestingly, predator presence did not alter subsidy effects on direct and indirect interactions within recipient food webs (predator presence main effect, p = 0.231; Table 1.2a).

Top-down effects of predators (i.e. predator effect, PE) significantly differed depending on the focal trophic level (PE trophic level main effect, p<0.001; Table 1.2b; Figure 1.1e), with the strongest top-down effects on primary producers in food webs receiving subsidies to primary producers (Figure 1.1a&e). There was no significant effect of subsidy trophic level or subsidy amount on PE (Table 1.2b). It is important to note that there was a large variation in both PE and SE effects on various trophic levels, with a range of positive and negative effect sizes (Figure 1.1a-c).

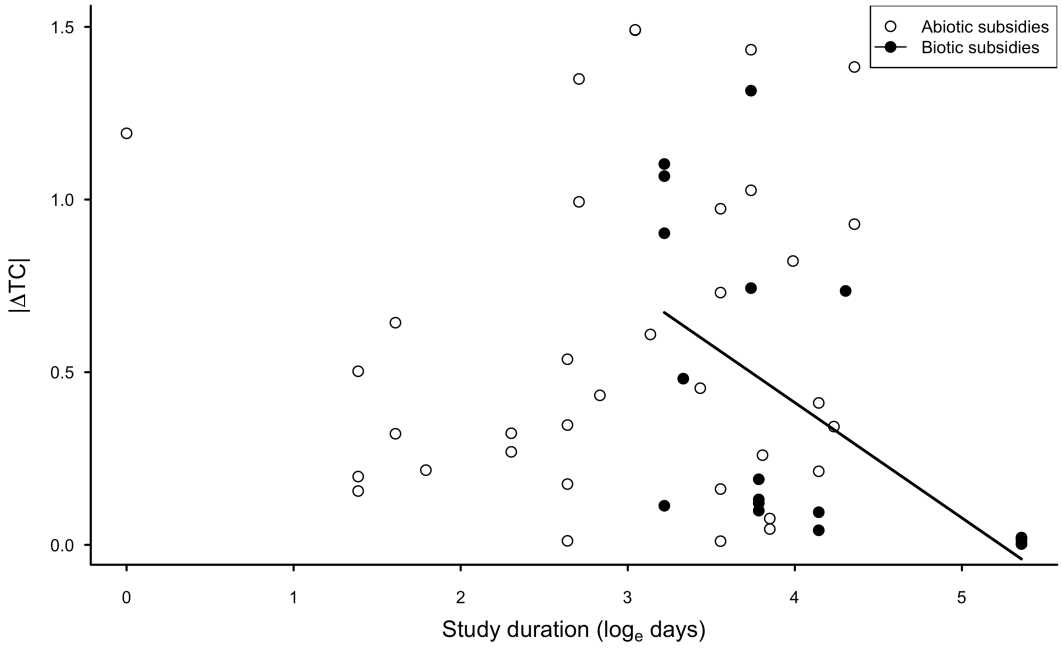


Figure 1.3: Change in trophic cascade strength between low and high subsidies, |ΔTC|, as predicted by study duration (log$_e$ days) for abiotic (subsidies to primary producers) and biotic (subsidies to primary consumers and predators) subsidies. Solid black line represents significant relationship for biotic subsidies between study duration and |ΔTC|.

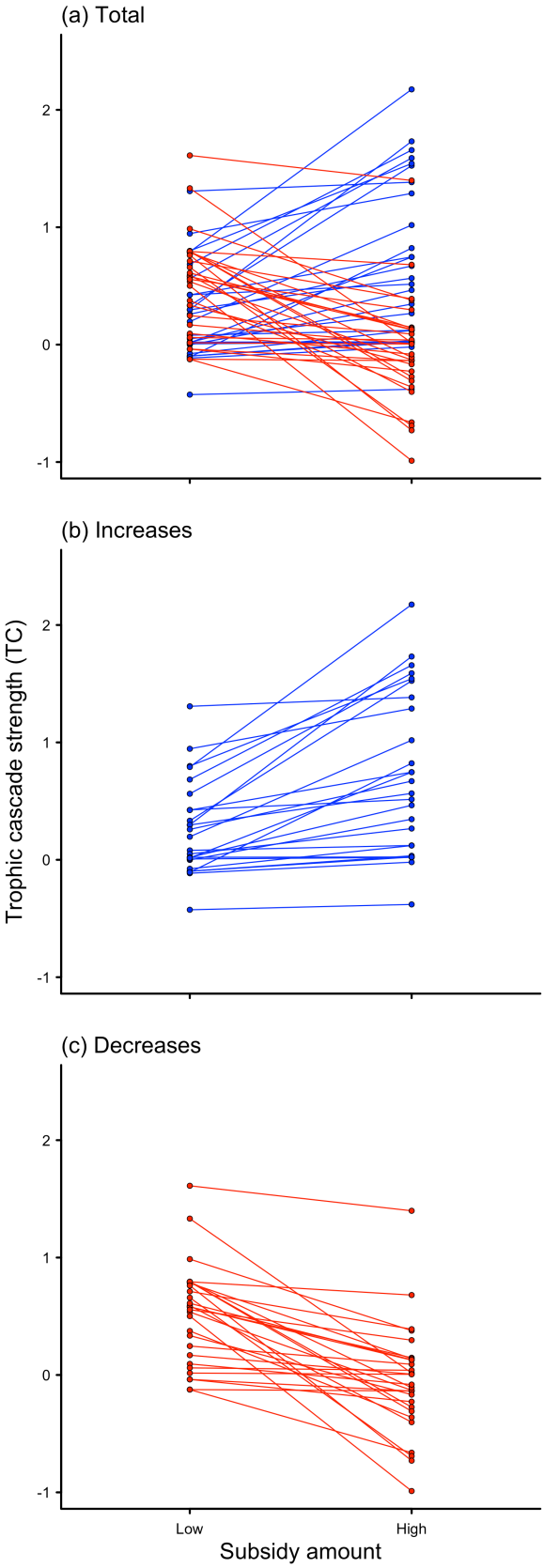


Figure 1.4: Change in trophic cascade strength at low and high subsidy levels within a data set for (a) all data sets (Total), (b) data sets where trophic cascade strength increased from low to high subsidies (blue points and lines); and (c) data sets where trophic cascade strength decreased from low to high subsidies (red points and lines).

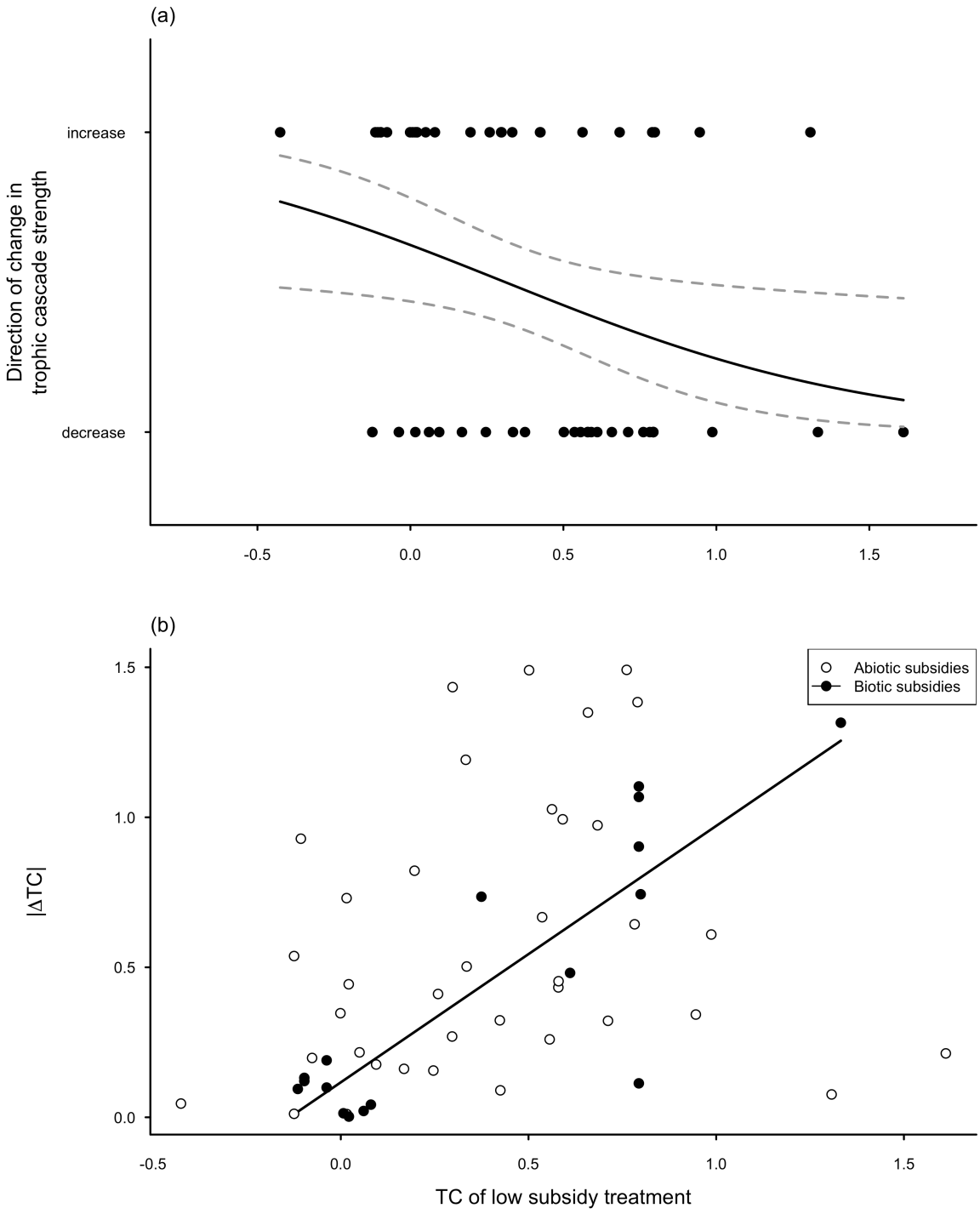


Figure 1.5: Correlation between TC (trophic cascade effect size) of low subsidy treatments and (a) whether trophic cascade strength decreased or increased from low to high subsidy amounts (predicted with a binomial generalised linear model), and (b) |ΔTC| (change in trophic cascade strength between low and high subsidies) for abiotic (subsidies to primary producers) and biotic (subsidies to primary consumers and predators) subsidies.

Trophic cascade strength

There were no significant effects of either subsidy trophic level or subsidy amount on top-down trophic cascades to primary producers (Table 1.2c; Figure 1.2). Interestingly, |ΔTC| could be predicted by study duration, with decreases in the difference in TC between high

and low subsides, with increased duration. However, this was only with biotic subsidies (subsidies to primary consumers and predators) and appears to be driven by only 2 data sets (study duration x abiotic/biotic subsidy type, p=0.035; Table 1.2d; Figure 1.3).

As a proxy for an unknown ecosystem property affecting the difference in TC between high and low subsidies (|ΔTC|), I investigated whether TC of low subsidy treatments could be used to predict the magnitude of difference in TC between low and high subsidy treatments (Figure 1.4). Low subsidy TC significantly predicted whether TC would increase or decrease with high subsidy input (TC of low subsidy treatment, p=0.018; Table 1.2e; Figure 1.5a). TC was more likely to increase from low to high subsidies if TC of low subsidy treatment was weak, whereas TC was more likely to decrease from low to high subsidies if the TC of low subsidy treatment was strong (Figure 1.4 & 1.5a). However, the relationship between TC of low subsidies and |ΔTC|, was only observed in studies with subsidies to primary consumers or predators (i.e. biotic subsidies; TC of low subsidy treatment x abiotic/biotic subsidy type, p=0.021; Table 1.2f; Figure 1.5b). Therefore, if TC of low subsidy treatments is strong, it is more likely that TC will decrease with high subsidy input and the magnitude of this change in trophic cascade strength will be large.

DISCUSSION

Subsidy effects on recipient consumers, as well as their indirect effect on whole food webs, are a hotly debated topic (Polis et al. 1997, Baxter et al. 2005, Marczak et al. 2007, Marcarelli et al. 2011, Bartels et al. 2012). Subsidy quality, quantity, and recipient ecosystem productivity have been proposed as an explanation driving variation observed within the subsidy literature (Marczak et al. 2007, Marcarelli et al. 2011, Klemmer and Richardson 2013). However subsidy trophic level and the mechanisms behind short- versus longer-term responses may account for the differences between studies (Huxel and McCann 1998, Huxel et al. 2002, Baxter et al. 2005, Leroux and Loreau 2008). The aim of this meta-analysis was to test whether subsidy trophic level affected bottom-up and top-down food web interactions, and to see if those effects changed through time. Subsidy trophic level and amount altered bottom-up interactions, with no effect on top-down interactions, most likely due to large variation in effect sizes. However, study duration and the trophic cascade strength (TC) in low subsidy treatments predicted the magnitude of TC difference due to low and high subsidy amounts, but only with subsidies to consumers and predators. Thus, this meta-analysis reveals that there are underlying variables, such as study duration and underlying TC of un-

manipulated systems, which are affecting the relationship between subsidies and trophic cascade strength.

Bottom-up and top-down food-web interactions

The strongest bottom-up effect in this meta-analysis was the direct increase in primary producers associated with subsidies to primary producers, which supports my original hypothesis, that subsidies would have positive direct effects on consumers. However subsidies to primary consumers and subsidies to predators did not have a positive direct effect on consumers. This is surprising given a meta-analysis by Marczak et al. (2007) found that subsidies positively increased consumer biomass/abundance. The variation that I found in direct bottom-up subsidy effects to consumers could be due to differences between short-term behavioural versus longer-term numerical effects. Shorter duration studies would not lead to a numerical increase in direct consumers of the subsidies, but instead elicit behavioural prey-switching between subsidies and *in situ* resources, whereas longer duration studies might see a numerical increase in direct consumers (as discussed in Baxter et al. 2005). Therefore, with a combination of both short and longer duration studies, there could potentially be high variation in bottom-up effects to consumers.

Study duration

The results from bottom-up subsidy effects and top-down predator effects suggest that study duration could potentially be leading to variable effects of subsidies within the published studies. For abiotic subsidies to primary producers (i.e. nutrient subsidies), I found that study duration did not predict the magnitude of subsidy effect on TC ($|\Delta TC|$). However, with biotic subsidies (subsidies to primary consumers and predators), longer study duration predicted lower $|\Delta TC|$. Similarly, a meta-analysis by Borer et al. (2005) found that study duration was correlated to trophic cascade strength, and when plant generation time was accounted for, study duration did not correlate with trophic cascade strength. Furthermore, terrestrial study duration was generally shorter than generation times of primary producers (and low trophic cascade values were reported), whilst aquatic study duration was longer than primary producer generation time (and higher trophic cascade values were reported) (Borer et al. 2005). In addition, there were multiple primary consumer and predator traits, such as metabolism, thermal regulation, and size, which were correlated with trophic cascade strength in the Borer et al. (2005) study. In my study, the differences between subsidy effects on trophic cascade strength may have be due to things such as study duration, primary

producer generation time, and primary consumer and predator characteristics, as discussed above. All of the contingencies due to system characteristics could alter whether study duration was long enough for numerical increases of consumers to propagate through to allow strong trophic cascades to be observed.

Multiple strategies can be incorporated in future studies on subsidy trophic level and trophic cascade strength to account for differences between study systems. First, consideration of generation times of all consumers within the food web could determine the study duration needed to see numerical effects. However, that strategy seems unlikely considering the long generation times within some focal systems, particularly terrestrial primary producers and long-lived vertebrate predators (Polis et al. 2000, Schmitz et al. 2000, Borer et al. 2005). Another possibility is, instead of manipulating predators and subsidies, to use natural gradients in subsidy input and predator presence that have experienced the numerical propagation, as demonstrated in Nyström et al. (2003). Lastly, future researchers can manipulate subsidy trophic level within one focal system to reduce variation due to system differences when investigating the effects of subsidy trophic level on trophic cascades.

Unknown experimental factors altering TC

I found TC of low subsidy treatments predicted the direction and magnitude of subsequent change (for biotic subsidies) in TC between low and high subsidy treatments. These correlations may indicate other differences between studies not addressed in my meta-analysis. First, it could suggest that systems with elevated TC due to low subsidy resources are stabilised with additional resources, and therefore increasing subsidies strongly decreases trophic cascades. Rooney et al. (2006) found that increasing detrital food-web compartments can stabilize food webs by increasing the diversity of strong and weak predator interactions. Therefore, it could be that some of the systems in this meta-analysis were stabilised with subsidies.

The correlation between low subsidy TC and the direction of change (i.e. increase or decrease) in TC between low and high subsidies may also be a reflection of how subsidies were manipulated in the study. Altering resources to a system through large additions (Huxel and McCann 1998), or removals of resources (Rooney and McCann 2012), can destabilise food webs. Therefore, the type of experimental manipulation (removal or addition) may lead to either an increase or decrease in trophic cascades as subsidy amount is manipulated from low to high subsidies. In particular, removals of subsidies can quickly alter feeding

relationships among trophic levels, leading to large increases in trophic cascade strength, as observed in Nakano et al. (1999). Therefore, strong TC with low subsidy treatments may correlate with large magnitude decreases in TC, from low to high subsidy amounts, due to subsidy removal creating a large disturbance, therefore increasing trophic cascades with low subsidy inputs.

Lastly, the relationship between TC of low subsidy treatments and direction and magnitude of change in TC between low and high subsidy values may be an artefact of bias within the studies used for the meta-analysis. There was low representation of terrestrial ecosystem studies (only 3 studies), and a majority of the data sets were from manipulation of abiotic nutrient subsidies to primary producers. Thus, more research into the effects of subsidies to primary consumers and subsidies to predators, especially in terrestrial ecosystems, is needed to understand how subsidy trophic level alters trophic cascade strength.

Conclusions

This meta-analysis revealed multiple knowledge gaps and suggests future directions for increasing understanding of how subsidies, and more specifically subsidy trophic level, alters top-down and bottom-up food-web interactions. First, further research on short- versus longer-term effects of subsidies on trophic interactions within individual study systems is needed. By understanding the relationship between short- versus long-term dynamics within one study system, better inferences can be made on the relationships of short- versus longer-term dynamics across systems. For example, opposed to comparing short-term dynamics of insect subsidies on stream ecosystems to longer-term dynamics of seaweed on beaches, we could be comparing the relationship between short- and longer-term dynamics of insect subsidies in streams to the relationship between short- and longer-term dynamics of seaweed on beaches.

Second, my meta-analysis revealed a lack of information on how subsidies entering at multiple trophic levels within one food web might affect food-web dynamics. Some systems naturally receive multiple types of subsidies that are consumed at different trophic levels, for example streams receiving terrestrial leaf subsidies (Nyström et al. 2003) and terrestrial insect subsidies (Nakano et al. 1999). However, little is known on the interaction between subsidies entering at multiple trophic levels (but see theoretical predictions by Huxel et al. 2002).

Last, there was little evidence regarding how manipulating subsidies going into a system will affect reciprocal subsidies out of the system (but see Baxter et al. 2004, Kraus and Vonesh 2012), and in particular how subsidy trophic level may affect subsidy exchange between highly connected ecosystems. Although our understanding of the relationship between subsidies and food-web interactions is growing, this meta-analysis highlights important gaps in subsidy research.

Photo: Angus McIntosh

Chapter Two: SUBSIDY TROPHIC LEVEL ALTERS FOOD-WEB STRUCTURE

INTRODUCTION

Increasing resources within food webs can create bottom-up cascades, where changes to consumer biomass ascend through food webs to alter top-predator biomass (Heath et al. 2014). While resources that vary along natural productivity gradients can increase the carrying capacity of food webs (Oksanen et al. 1981), resources originating outside of an ecosystem (i.e. cross-ecosystem subsidies) can also create strong bottom-up interactions by increasing consumer biomass above what can be supported by *in situ* production (Polis et al. 1997, Marczak et al. 2007, Bartels et al. 2012). The trophic level at which a subsidy enters an ecosystem can also influence food web structure and dynamics (Polis et al. 1997, Richardson et al. 2010, Bartels et al. 2012). In particular, subsidies to predators enter at the top of food webs, and by increasing predator biomass, can have consequences for biomass at lower trophic levels through top-down processes (Henschel et al. 2001). In comparison, subsidies to primary consumers enter at the bottom of food webs and their effects can lead to bottom-up increases in consumer biomass which subsequently alter community structure (Wallace et al. 1997, Nyström et al. 2003). Although these processes are well documented in isolation (Marczak et al. 2007, Marcarelli et al. 2011, Bartels et al. 2012, Hagen et al. 2012a), little is known about how these two types of cross-ecosystem resources interact to alter food-web regulation.

Cross-ecosystem subsidies to predators can increase predator abundance/biomass and alter predatory interactions with *in situ* prey (Nakano et al. 1999, Henschel et al. 2001, Sato et al. 2012). Subsidies to predators include emerging aquatic insects to terrestrial spiders and birds (Henschel et al. 2001, Collier et al. 2002, Murakami and Nakano 2002, Marczak and Richardson 2007), terrestrial arthropods falling into freshwater ecosystems consumed by fish (Nakano et al. 1999, Baxter et al. 2004, Sato et al. 2012), or marine organisms that provide resources to large terrestrial predators (Rose and Polis 1998, Bouchard and Bjorndal 2000, Roth 2003). These subsidies to predators can induce behavioural prey-switching in predators, where they feed on subsidies as opposed to *in situ* prey, resulting in numerical increases in *in situ* prey (Nakano et al. 1999, Sabo and Power 2002). However, continued input of resource subsidies will likely lead to a numerical increase in either abundance or biomass of predators

(Henschel et al. 2001). When predators either become satiated or are absent from the system, subsidies normally consumed by predators can also enter detrital food-web pathways, thereby becoming subsidies to primary consumers and detritivores (Nowlin et al. 2007, Hoekman et al. 2011, Dreyer et al. 2012). Thus, the trophic level at which a subsidy enters a food web also depends on the presence and feeding rate of consumers within recipient ecosystems.

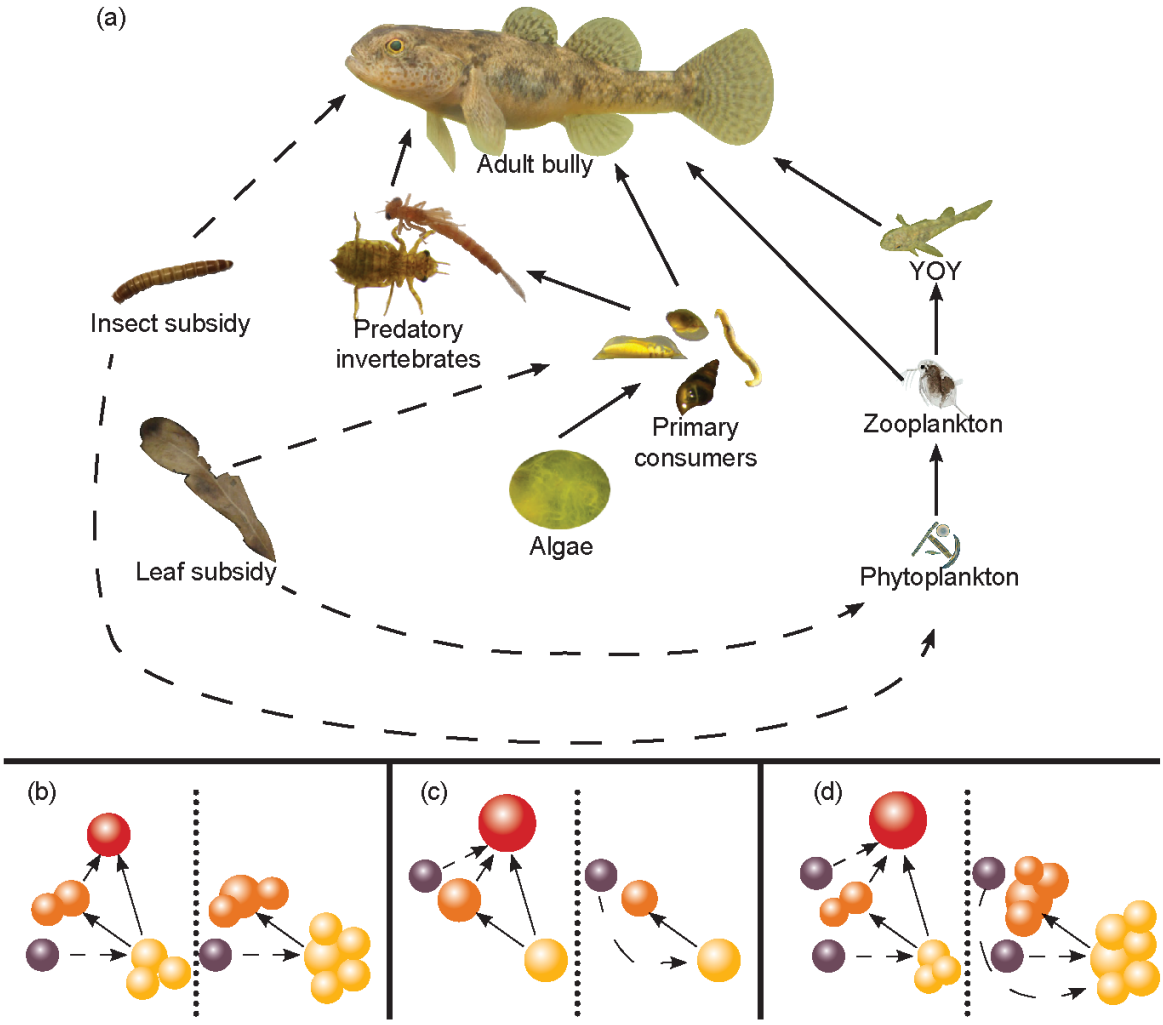


Figure 2.1: : Depiction of proposed bottom-up energy movement (a) in the freshwater food web used in the mesocosm experiment, and proposed effects of (b) subsidies to primary consumers, (c) subsidies to top-predators, and (d) both kinds of subsidies to primary consumers and top-predators on food webs with and without top-predators. Only the main groups of taxa identified within the food webs are represented in these diagrams (YOY: young of year fish). Solid arrows represent interactions between aquatic organisms and dashed arrows represent interactions between terrestrial resources and aquatic organisms. Red spheres, top-predators; dark orange spheres, intermediate predators; light orange spheres, primary consumers; purple spheres, subsidies; number of spheres, taxa richness; size of spheres, biomass; dashed lines, subsidy interactions; solid lines, *in situ* resource interactions; and dotted lines in b-d indicate situation with (left) and without (right) top predators.

Subsidies to primary consumers include terrestrial leaves that fall into freshwater ecosystems (Wallace et al. 1997, Nyström et al. 2003, Klemmer and Richardson 2013), marine seaweed that washes onto beaches (Polis and Hurd 1996, Dugan et al. 2003, Piovia-Scott et al. 2011), and algae exposed to terrestrial consumers as freshwater ecosystems dry (Bastow et al. 2002). Similar to subsidies to predators, subsidies to primary consumers can increase consumer biomass (Hagen et al. 2012a). However, they can also alter food-web structure by creating a heterogeneous resource base supporting many types of primary consumers, and thereby creating food-web compartments (i.e. subsets of taxa that interact more strongly with each other than others within the food web; Moore et al. 2004). Subsidies, like terrestrial leaves to freshwater food webs, break down into fine particulate organic matter (FPOM), dissolved organic matter (DOM), and nutrients, which can create horizontal (within-trophic level) food-web diversity by supporting different functional feeding groups of primary consumers (Wallace et al. 1997, Dugan et al. 2003, Moore et al. 2004). The breakdown of subsidies to primary consumers can also alter multiple food-web compartments, such as benthic and pelagic compartments in lentic freshwater ecosystems. For example, nutrients and DOM from the breakdown of terrestrial leaves can increase pelagic phytoplankton and zooplankton biomass in lakes (Cottingham and Narayan 2013). Although subsidies to primary consumers increase biomass and diversity at the base of food webs, their effects can vertically cascade up the food web increasing predator abundance/biomass (Polis and Hurd 1995). Therefore, subsidies to primary consumers have the potential to alter vertical and horizontal food-web structure (Hagen et al. 2012a).

The aim of this study was to investigate how subsidies entering at the top and bottom of food webs alter bottom-up changes to consumer biomass and food-web structure. I ran an 18-month, freshwater pond, mesocosm experiment factorially manipulating input of terrestrial insect subsidies, input of terrestrial leaf subsidies, and presence of top-predators (fish; Figure 2.1a). Firstly, I predicted that leaf subsidies would increase consumer biomass, alter consumer composition, and create resources for pelagic food-web compartments (Figure 2.1a&b). I also expected that insect subsidies would increase consumer biomass throughout the food web, via direct consumption, but fish presence would decrease biomass and diversity of lower trophic levels (Figure 2.1c). Lastly, I hypothesised that the addition of both leaf and insect subsidies together would lead to large additive biomass increases in all trophic levels, as well as altered community composition of predatory invertebrates and primary consumers (Figure 2.1d).

METHODS

Experimental set-up

The experiment took place in the Southern Alps on South Island, New Zealand at the University of Canterbury's Cass Mountain Research Station. In January 2013, thirty-two 1,100-litre cattle tanks (mesocosms) were filled with ground water, 1 cm of gravel substrate, common pond macrophytes from the area (submergent *Myriophyllum* and emergent *Carex*), and two 10-cm diameter ceramic pots positioned on their side to provide fish habitat. Experimental tanks were inoculated with 5 l of filtered pond water, 380-ml aliquots of concentrated phytoplankton and zooplankton, and 300 ml filtered fine particulate organic matter. To ensure representation of various trophic levels and feeding groups, a range of benthic invertebrates from local ponds and lakes were added to tanks in natural densities based on exploratory surveys (predatory invertebrates: 10 *Procordulia* dragonflies and 10 *Xanthocnemis* damselfies; primary consumers: ~200 *Potamopygrus* snails, ~100 Chironominae midges and 50 *Triplectides* caddisflies). In addition to those taxa, two 0.3 m^2 sweeps of benthos with a 1-mm mesh D-net from local ponds (one from a permanent pond and one from a temporary pond) were added to the tanks to increase the diversity of rare taxa and mimic naturally occurring food webs. Tanks were left to be naturally colonised by terrestrial dispersal of adult stages of invertebrates from January to May 2013 before experimental treatments were added; natural colonization continued to occur throughout the duration of the experiment.

The experiment had a fully crossed 2 x 2 x 2 factorial design, with the presence/absence of subsidies to primary consumers (leaves) and the presence/absence of subsidies to top-predators (beetle larvae) crossed with the presence/absence of top-predators (fish). Each treatment was replicated four times and randomised within four spatial blocks of tanks. Ten adult upland bullies (*Gobiomorphus breviceps*; totalling 26.6 +/- 0.4 g as mean +/- 1 standard error), a common native predatory fish in New Zealand freshwater ecosystems (Staples 1975), were added as the top predator to "fish" treatment tanks on 14 May 2013. These adult fish reproduced in December 2013, and young-of-the-year (YOY) upland bullies were present from this point onward.

Four grams of air-dried riparian willow leaves (*Salix fragilis,* most common riparian tree in the area) collected from the area were added as resources to primary consumers ("leaf subsidy") every four weeks, beginning 22 May 2013. Every two weeks, beginning 22 May 2013, two grams of live beetle larvae (lab cultured *Tenebrio* sp.) were added as resources to

predatory fish ("insect subsidy"). Beetle larvae were used so I could easily control subsidy additions and to maximize the ability to detect their assimilation into the food web through stable isotopes.

Food-web sampling

Five mesh sampling baskets (0.04 m^2) containing cobble and fabric cut to mimic leaves were placed in the tank as sampling devices for invertebrates > 5.0 mm in length. At the conclusion of the experiment, on 3 November 2014, one basket was removed from each tank and all invertebrates were separated from the basket and preserved in ethanol for later identification. Tanks were then destructively sampled to measure fish and large invertebrate biomass. This involved a combination of basket (invertebrates of body length < 5.0 mm) and whole tank sampling (for invertebrates > 5.0 mm) for final invertebrate biomass estimates in mg/m^2.

I used electric fishing to remove adult and YOY bullies from the experimental tanks. This was done as quickly as possible to prevent gut content loss. Once stunned, fish were removed with aquarium nets from the tanks and euthanized in a bucket of water containing a lethal dose of 2-phenoxyethanol (1.0-2.0 mL/L). Euthanized fish were then weighed, measured, and dissected of foreguts (preserved in 90% ethanol) before being frozen.

To sample all remaining invertebrates, five-minute D-net (1-mm mesh) sweeps were conducted by two people simultaneously within the same tank. The tank water then was emptied by bucketing into 1-mm mesh D-nets until there was approximately 10 cm of water remaining. This was followed by two sweeps of tanks with the D-nets. All macro-invertebrates where removed from these samples directly after sampling using large sorting trays and a portion were frozen for stable isotope analysis with the rest being preserved in 70% ethanol. Samples of algae and intact willow leaf subsidies were also frozen for stable isotope analysis.

In the laboratory, all invertebrates > 0.5 mm were identified to lowest taxonomic unit, usually genus or species using Winterbourn et al. (2006) and categorized into primary consumers and predatory invertebrates, following Greig (2008). They were then photographed using a Leica DFC450 microscope camera. Length measurements were taken by uploading the digital photographs to Adobe Acrobat Pro and measuring the invertebrates using the 'Measuring Distance' tool. Dry weights of invertebrates were calculated using length-weight regressions (Towers et al. 1994, Nyström and Pérez 1998, Benke et al. 1999, Baumgärtner and Rothhaupt 2003, Stoffels et al. 2003, Greig 2008). Invertebrates were

categorized as either predatory invertebrates or primary consumers, but primary consumer invertebrates were not separated further into algivores or detritivores, because most species were facultative consumers of both types of resources (Stark 1981, Winterbourn et al. 1981, Winterbourn 2000). To estimate final invertebrate biomass (mg/m^2), a combination of basket (invertebrates of body length < 5.0 mm) and whole-tank sampling (invertebrates > 5.0 mm) was used.

In the laboratory, frozen fish and invertebrates were thawed for stable isotope processing. Invertebrates where rinsed with purified milli-q water to remove algae and other debris. Caddisfly larvae and snails were removed from their cases or shells to avoid contamination by non-dietary carbon. Guts were removed from predatory invertebrates to avoid contamination from ingested prey (Jardine et al. 2005). Whole invertebrates (sometimes multiple individuals depending on taxa), dissected dorsal muscle tissue from fish, algae, willow leaves, and insect subsidies (beetle larvae) were dried (60°C for > 48 hours), then ground to a fine, homogenous powder, using a mortar and pestle and weighed (1+/- 0.15 mg for animals or 3 +/- 0.3 mg for plants). Weighed aliquots were encapsulated within aluminium tins and analysed at the University of California Davis Stable Isotope Facility on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon LTD., Cheshire, UK).

Isotope ratios were calculated as:

$$\delta X = (R_1/R_0 - 1) \times 1000$$

where X is either 13C or 15N, R_1 the ratio of 13C/12C or 15N/14N of the samples, and R_0 the ratio of 13C/12C standard of Vienna PeeDee Belemnite or 15N/14N of standard of atmospheric N_2. The precision of analysis was between 0.06 and 0.10‰ for δ13C and between 0.09 and 0.14‰ for 15N based on standard deviation of laboratory standards (Bovine liver, USGS-41 Glutamic Acid, Nylon 5, and Peach leaves). δ13C was post-corrected for lipids using mathematical normalizations from Post et al. (2007).

Statistical analysis

Biomass of adult (g of wet weight per tank) and YOY (g of wet weight per tank) bullies, was analysed using mixed effects models with presence/absence of leaf subsidies and presence/absence of insect subsidies as fixed factors, and experimental block as a random factor. Biomass of predatory invertebrates (dry weight mg/m^2) and primary consumers (dry weight mg/m^2) were analysed using mixed effects models with presence/absence of leaf subsidies, presence/absence of insect subsidies, and presence/absence of fish as fixed factors,

and experimental block as a random factor. Post-hoc mixed effects models for predatory invertebrate and primary consumer biomass were run separately for fish and fishless food webs, with presence/absence of leaf subsidies and presence/absence of insect subsidies as fixed factors, and experimental block as a random factor. All biomass measurements were $\log_e$-transformed to meet assumptions of normality. Mixed-effects models were run using the nlme package (Pinheiro et al. 2015) in R (R Core Team 2014).

The isotopic niches of predatory invertebrates and primary consumers were analysed using δ13C and 15N range (Layman et al. 2007), and with Bayesian ellipse metrics (Jackson et al. 2011). δ13C and 15N ranges were calculated as the difference between the maximum and minimum values for the respective stable isotope ratios and were used to determine either a change in trophic diversity (15N range) or a change in basal resource diversity (13C range; Layman et al. 2007). I used Bayesian ellipse metrics, rather than convex hull area, to estimate the isotope niche width within the δ13C and δ15N bi-plot space, because these ellipse metrics are less sensitive to sample size (Jackson et al. 2011). Ellipse area/size increases could indicate both a change in consumption of resources by a given taxon (altered C13 and 15N of individuals) and increase in the number of taxa. Ellipse area and theta (slope of semi-major ellipse axis) were calculated using the mean of 10,000 posterior draws corrected for small sample size, with tank as a grouping variable, using the siber.ellipse and standard.ellipse functions from the SIAR package in R (Parnell et al. 2008). The size of ellipse area is an estimator of isotopic niche width, which combines trophic height (15N) and carbon breadth (δ13C), and theta is an estimator of the relative contributions of δ13C and 15N to that niche width; high theta values equate to 15N contributing more to ellipse area and low theta values mean δ13C contributes more to ellipse area (Jackson et al. 2011). The absolute value of theta was used because the magnitude, not direction, of the slope determines δ13C and 15N contribution to ellipse area. Mixed-effects models (nlme package; Pinheiro et al. 2015) were used to analyse the contribution of terrestrial leaf subsidies, terrestrial insect subsidies, as well as fish presence (all fixed factors), on the above isotope niche metrics (δ13C and 15N range, ellipse area, and ellipse theta) with experimental block as a random factor.

To test the effects of leaf subsidies, insect subsidies, and fish presence on invertebrate community composition, I used non-metric multidimensional scaling (NMDS) ordination to summarise community structure. I chose this over parametric ordinations (e.g. RDA) to avoid making assumptions regarding normality of the data and to base analyses on dissimilarity indices that are more ecologically meaningful (Minchin 1987, Anderson et al. 2011). Modified Gower dissimilarity indices, to reflect differences in both composition and

abundance (Anderson et al. 2011), were calculated on $\log_e(+1)$-transformed biomass data of invertebrates with experimental tanks as replicates. I tested for differences between treatments (terrestrial leaf subsidies, terrestrial invertebrate subsidy, and fish presence) and their interactions using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations using the adonis function in the vegan package (Oksanen et al. 2011) in R (R Core Team 2014).

Table 2.1: Results of mixed-effects linear models, as probability p-values, with insect subsidies (I), terrestrial leaf subsidies (L), and fish presence (F) as fixed factors, experimental block as a random factor, for (a) adult bully total mass (g of wet weight/tank), (b) young of year bully (YOY) total mass (g of wet weight/tank), predatory invertebrate (c) biomass (mg/m^2), (d) δ13C range, (e) δ15N range, and (f) siber ellipse area and theta (slope of semi-major ellipse axis) of δ13C and δ15N isotopes, and primary consumer (g) biomass (mg/m^2), (h) δ13C range, (i) δ15N range, and (j) siber ellipse area and theta (slope of semi major ellipse axis) of δ13C and δ15N stable isotopes. Also p-values from a (NMDS) adonis model (k) on invertebrate taxa biomass (mg/m^2) are shown. P-values are bolded when <0.050. Dashes represent predictor variables that were not included in the model.

Response variable	**Predictor variables**						
	I	**L**	**F**	**IxL**	**IxF**	**LxF**	**IxLxF**
Adult bullies							
(a) Biomass* (g/tank)	**<0.001**	0.175	-	0.979	-	-	-
YOY bullies							
(b) Biomass* (g/tank)	**0.022**	**0.021**	**-**	0.231	-	-	-
Predatory invertebrates							
(c) Biomass* (mg/m^2)	**0.044**	**0.006**	**<0.001**	0.331	0.945	0.196	0.311
(d) 13Carbon range	0.969	0.405	**<0.001**	0.421	0.902	0.205	0.643
(e) 15Nitrogen range	0.598	0.626	0.263	0.658	0.547	0.212	0.176
(f) Siber ellipse area	0.679	0.489	**<0.001**	0.599	0.604	0.196	0.728
Theta (absolute value)	0.722	0.831	**<0.001**	0.669	0.759	0.113	0.710
Primary consumers							
(g) Biomass* (mg/m^2)	0.752	0.689	**0.001**	0.148	0.870	0.083	0.343
(h) 13Carbon range	0.212	0.347	**0.004**	0.407	0.121	0.256	**0.024**
(i) 15Nitrogen range	0.296	**0.004**	**<0.001**	0.058	0.409	0.060	0.729
(j) Siber ellipse area	**0.038**	**0.008**	0.753	0.459	0.232	**0.036**	0.385
Theta (absolute value)	0.649	0.852	**0.005**	0.053	0.365	0.417	0.323
Invertebrates (mg/m^2)**							
(k) ParMANOVA	0.186	**0.048**	**0.001**	0.617	0.859	0.069	0.42

* Indicates $\log_e$-transformed response variable

** Indicates (y+1) $\log_e$-transformed response variable

RESULTS

Biomass

Insect subsidies significantly increased adult fish biomass, with some tanks containing more than double the fish mass of tanks receiving just leaf subsidies (fish main effect, $p<0.001$; Figure 2.2a; Table 2.1a). Leaf subsidies and the interaction between leaf and insect subsidies did not significantly affect adult fish mass (leaf main effect, $p=0.175$; leaf x insect interaction, $p=0.979$; Table 2.1a). Leaf and insect subsidy additions significantly increased YOY fish mass (leaf main effect, $p=0.021$; insect main effect, $p=0.022$; Table 2.1b; Figure 2.2b), however there was not a significant interaction (insect x leaf interaction, $p=0.231$; Table 2.1b), indicating independent effects of subsidies.

Fish significantly decreased predatory invertebrate biomass in the tanks (fish main effect, $p<0.001$; Table 2.1c ; Figure 2.2c&d), which was likely due to a large, 2-fold decreases in the biomass of *Procordulia grayi* dragonfly larvae when fish were present (Table 2.2). In addition to the fish effect, leaf and insect subsidies significantly increased predatory invertebrate biomass (leaf main effect, $p=0.006$; insect main effect, $p=0.044$; Table 2.1c; Figure 2.2c&d), again likely due to large increases in dragonfly larvae biomass (Table 2.2). Overall, dragonfly larvae constituted between 46-68% and 30-76% of total invertebrate biomass in fishless and fish treatments, respectively. There were no significant interactions between leaf subsidy, insect subsidy, and fish treatments affecting predatory invertebrate biomass (Table 2.1c).

Primary consumer biomass significantly decreased in fish tanks (fish main effect, $p=0.001$; Table 2.1g; Figure 2.2e&f). In tanks with fish, the majority of primary consumer biomass was made up of *Potamopyrgus* snails, *Paroxyethira* caddisfly larvae, and Chironominae larvae, with the addition of *Physa* snails in fishless tanks (Table 2.2). Interestingly, there were no significant effects of leaf subsidies, insect subsidies, or their interactions on primary consumer biomass (Table 2.1g).

Table 2.2: Mean biomass (mg/m^2 dry weight) of invertebrate taxa identified in fish and fishless tanks after 532 days of receiving either no terrestrial subsidies (N), terrestrial leaf subsidies (L), terrestrial insect subsidies (I), or both terrestrial leaf and insect subsidies (LxI). Values in parentheses represent 1 standard error of the mean (S.E.). Dashes indicate that the taxon was not identified in tanks of that treatment after 532 days. All taxa were aquatic larval stages unless noted with (A) for aquatic adult life stages

	No fish				Fish			
Taxa	**N**	**L**	**I**	**LxI**	**N**	**L**	**I**	**LxI**
Acari	1.07 (0.78)	0.51 (0.51)	4.74 (4.74)	0.35 (0.35)	2.92 (2.75)	16.76 (7.02)	24.99 (14.93)	27.58 (8.66)
Anisops sp.	37.62 (18.61)	168.75 (33.58)	87.99 (42.67)	276.03 (103.95)	0.69 (0.69)	0.85 (0.85)	- -	9.78 (9.78)
Antiporus sp. (A)	- -	0.31 (0.31)	0.31 (0.31)	0.94 (0.60)	- -	- -	- -	- -
Austrolestes sp.	- -	- -	0.47 (0.47)	1.41 (1.41)	- -	- -	- -	- -
Ceratopogonidae	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
Chironominae	13.74 (6.60)	31.84 (14.52)	60.15 (16.78)	68.28 (37.37)	20.85 (9.25)	23.12 (10.83)	24.56 (9.81)	29.77 (6.39)
Diaprepocoris sp.	3.48 (2.13)	- -	4.42 (2.40)	19.02 (10.58)	- -	- -	- -	- -
Diptera pupae	0.44 (0.44)	0.40 (0.27)	5.13 (3.99)	2.31 (0.38)	1.46 (0.88)	- -	3.72 (2.11)	6.75 (6.46)
Gyraulus sp.	1.99 (1.99)	48.70 (30.84)	32.76 (29.59)	5.60 (4.85)	1.16 (1.16)	- -	0.06 (0.06)	0.03 (0.03)
Hudsonema sp.	<0.01 <0.01	- -	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	- -	<0.01 <0.01
Liodessus sp. (A)	0.77 (0.41)	0.38 (0.05)	0.27 (0.14)	0.44 (0.18)	- -	- -	0.05 (0.05)	- -
Oligochaeta	0.80 (0.25)	1.40 (0.22)	1.49 (0.32)	0.61 (0.16)	1.50 (0.58)	0.13 (0.08)	1.55 (1.11)	1.14 (0.65)
Olinga sp.	- -	0.04 (0.04)	0.43 (0.43)	- -	- -	- -	- -	2.02 (1.17)
Orthocladiinae	- -	1.07 (0.62)	2.59 (1.97)	3.15 (2.34)	6.05 (1.34)	0.50 (0.50)	4.33 (1.94)	1.06 (0.62)
Paroxyethira sp.	29.55 (15.31)	26.67 (9.79)	86.96 (48.54)	25.79 (22.15)	57.88 (34.41)	73.07 (55.72)	82.85 (30.35)	8.68 (4.94)
Physella sp.	72.26 (63.24)	561.70 (365.73)	281.81 (273.20)	117.68 (65.03)	- -	- -	- -	- -
Planorbidae	- -	2.15 (2.15)	6.15 (3.61)	3.33 (3.33)	1.88 (1.88)	- -	- -	- -
Potamopyrgus sp.	362.32 (230.92)	499.31 (164.65)	324.90 (59.49)	288.60 (108.42)	165.32 (46.46)	77.15 (45.30)	162.58 (32.72)	129.79 (69.91)
Procordulia grayi	810.98 (302.31)	1323.18 (521.18)	1093.97 (169.02)	2001.78 (671.69)	136.32 (39.27)	800.04 (183.50)	607.77 (192.98)	909.36 (354.34)
Procordulia smithi	- -	- -	9.25 (9.25)	7.96 (7.96)	8.04 (8.04)	10.00 (6.83)	9.15 (9.15)	32.63 (21.05)
Rhantus sp. (A)	40.25 (15.41)	42.93 (19.10)	29.51 (8.05)	34.88 (20.73)	- -	- -	- -	- -
Rhantus sp.	- -	0.03 (0.03)	0.25 (0.17)	0.83 (0.83)	- -	- -	- -	- -
Sigara sp.	0.56 (0.56)	14.57 (6.58)	4.37 (2.16)	21.39 (6.52)	- -	0.01 (0.01)	- -	0.03 (0.03)
Sphaeriidae	2.44 (2.44)	10.78 (5.14)	3.04 (1.82)	0.69 (0.69)	1.12 (1.12)	0.72 (0.72)	24.26 (24.26)	- -
Tanypodinae	23.84 (9.21)	48.47 (26.44)	114.39 (44.39)	36.09 (7.44)	28.36 (9.14)	27.11 (8.30)	41.70 (21.88)	41.80 (20.39)
Triplectides sp.	7.18 (7.18)	3.23 (1.45)	1.24 (1.24)	13.30 (13.30)	- -	- -	0.28 (0.28)	- -
Xanthocnemis sp.	25.46 (10.44)	36.94 (19.88)	30.05 (15.93)	33.18 (9.02)	8.01 (3.65)	15.93 (8.40)	6.85 (1.77)	10.78 (6.70)
Total	**1435 (273)**	**2823 (246)**	**2187 (340)**	**2962 (700)**	**442 (92)**	**1045 (130)**	**995 (255)**	**1211 (400)**

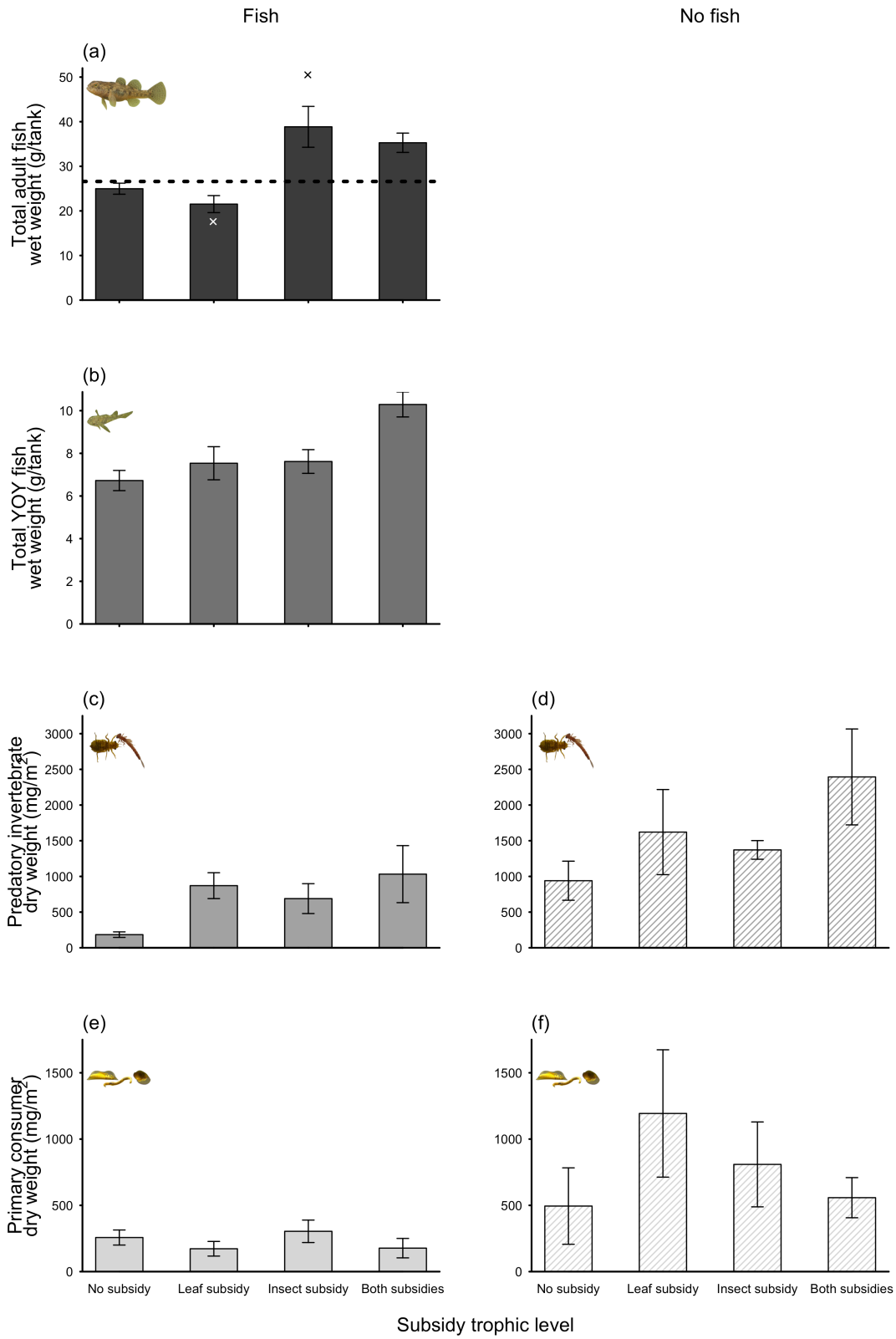


Figure 2.2: : Biomass of (a) adult bullies (g of wet weight/tank), (b) young-of-year (YOY) bullies (g of wet weight/tank), predatory invertebrates (mg/m^2) in tanks with (c) and without fish (d), and primary consumers (mg/m^2) in tanks with (e) and without fish (f) receiving no terrestrial subsidies, terrestrial leaf subsidies, terrestrial insect subsidies, and both terrestrial leaf and insect subsidies. The dashed line in (a) is starting fish wet weight and the "x"s are the lowest and highest fish weights. Error bars are +/- 1 standard error of the mean.

Invertebrate isotopic niche

Fish significantly decreased the δ13C range of predatory invertebrates (fish main effect, $p<0.001$), but had no significant effect on their δ15N range (fish main effect, $p=0.263$; Figure 2.3; Table 2.1d&e). Similar results were found using Bayesian ellipses, with fish significantly decreasing ellipse size (fish main effect $p<0.001$) and increasing ellipse theta (fish main effect, $p<0.001$; Table 2.1f; Figure 2.3), indicating the carbon range of predatory invertebrates was restricted by fish. Neither leaf nor insect subsidies had significant effects on δ13C range, δ15N range, ellipse size, or ellipse theta of predatory invertebrates (Table 2.1d-f). Thus, these isotopes indicate the major dietary and compositional influence on predatory invertebrates was the presence of fish that restricted their diet composition and breadth.

There was a significant leaf x insect x fish interaction affecting primary consumer δ13C range, probably because fish decreased primary consumer δ13C range with no subsidies, leaf subsidies, and both leaf and insect subsidies, but increased δ13C range with just insect subsidy input (insect x leaf x fish interaction, $p=0.024$; Table 2.1h; Figure 2.3). Primary consumer δ15N range significantly decreased with fish (fish main effect, $p<0.001$) and significantly increased with leaf subsidies (leaf subsidy main effect, $p=0.004$; Table 2.1i; Figure 2.3). There was no significant effect of insect subsidies on primary consumer δ13C or δ15N range (Table 2.1h&i). Insect subsidies decreased primary consumer ellipse area (insect main effect, $p=0.038$), while leaf subsidies significantly increased ellipse area (leaf main effect, $p=0.008$; Table 2.1j; Figure 2.3). Fish decreased primary consumer ellipse area, but only with leaf subsidies (fish x leaf interaction, $p=0.036$; Table 2.1j; Figure 2.3). Fish significantly decreased ellipse theta (fish main effect, $p=0.005$; Table 2.1j), indicating decreased δ15N contribution to ellipse area with fish.

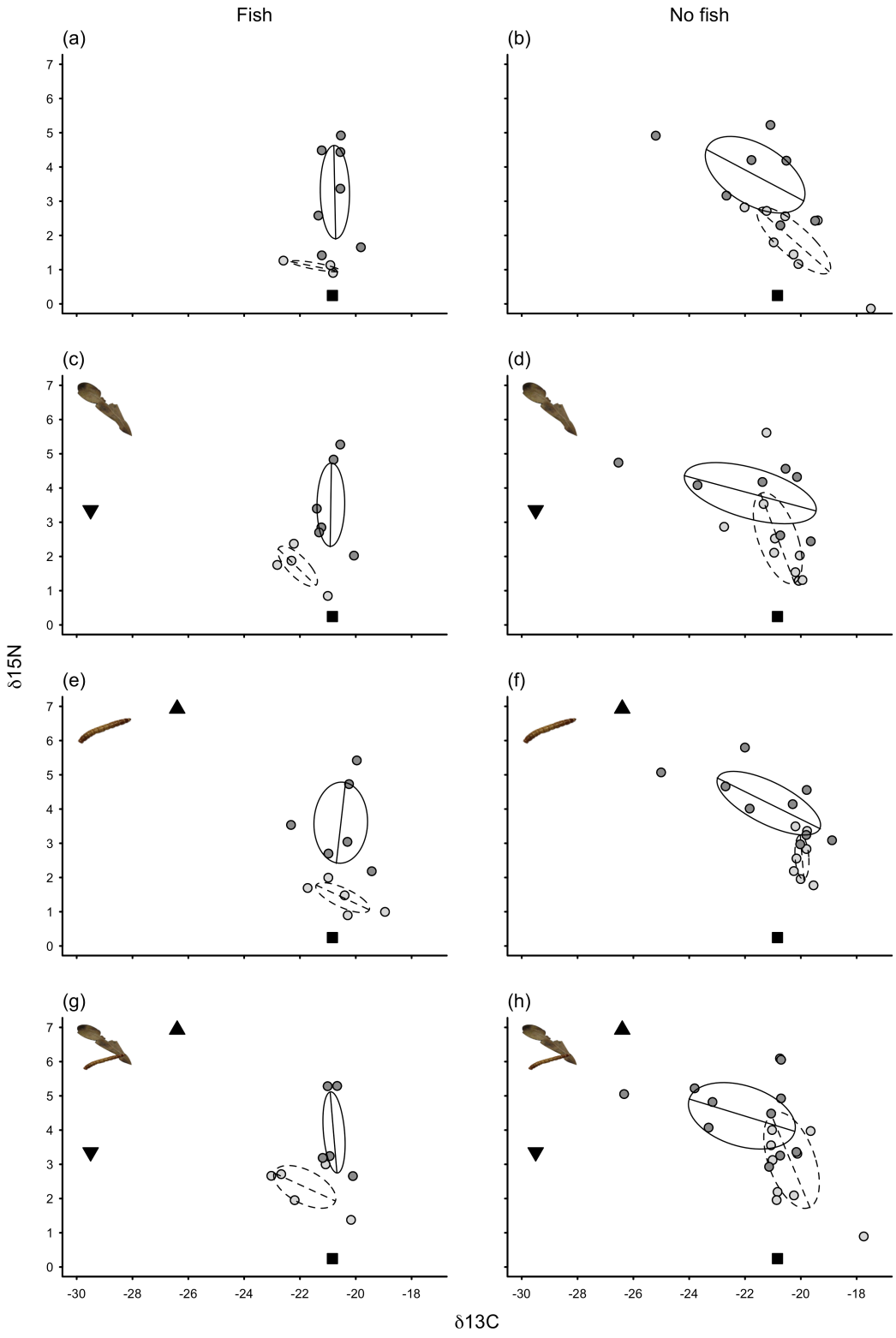


Figure 2.3: Delta 13 carbon (δ13C) and delta 15 nitrogen (δ15N) of predatory invertebrates (dark grey points and solid lines) and primary consumers (light grey points and dashed lines) in fish and fishless tanks receiving no terrestrial subsidies (a & b), terrestrial leaf subsidies (c & d), terrestrial insect subsidies (e & f), and both terrestrial leaf and insect subsidies (g & h). Black squares are the mean algal δ13C and δ15N signatures. Black downward-facing triangles are mean terrestrial willow leaf δ13C and δ15N signatures. Black upward-facing triangles are mean terrestrial insect δ13C and δ15N signatures. Ovals represent 999 permutated ellipses corrected for small sample size and lines represent the slope of semi-major axis of the ellipse (theta).

Invertebrate community composition

Analysis of community composition with PERMANOVA indicated significant fish and leaf subsidy effects (fish main effect, p=0.001; leaf main effect, p=0.048; Table 2.1k; Figure 2.4). In particular, predatory invertebrate taxa differed greatly between fish and fishless food webs. All beetle adults and larvae (*Rhantus* and *Antiporus*) were absent from fish tanks (Figure 2.4; Table 2.2), with the exception of a single *Liodessus* beetle adult found in a fish tank receiving insect subsidies (Table 2.2). Interestingly, *Physa* snails were also absent from fish tanks (Figure 2.4; Table 2.2). As previously stated, predatory dragonfly larvae biomass was halved in fish tanks, but biomass of other predatory invertebrates, such as *Xanthocnemis* damselfly larvae and the pelagic predator, *Anisops* backswimmers, was also reduced. Backswimmers, however, increased in biomass in leaf subsidy treatments (Table 2.2). Tanks receiving leaf subsidies had increased *Olinga* and *Triplectides* caddisfly larvae (a major detritivore in the system) biomass, but showed no change in *Paroxyethira* caddisfly larvae biomass. Orthocladiinae chironomid biomass was lowest in leaf subsidy treatments, in both fish and fishless food webs (Figure 2.4; Table 2.2). Although insect subsidies did not significantly alter community composition in the PERMANOVA model, fishless tanks receiving insect subsidies had the highest taxa richness of all treatments (Table 2.2).

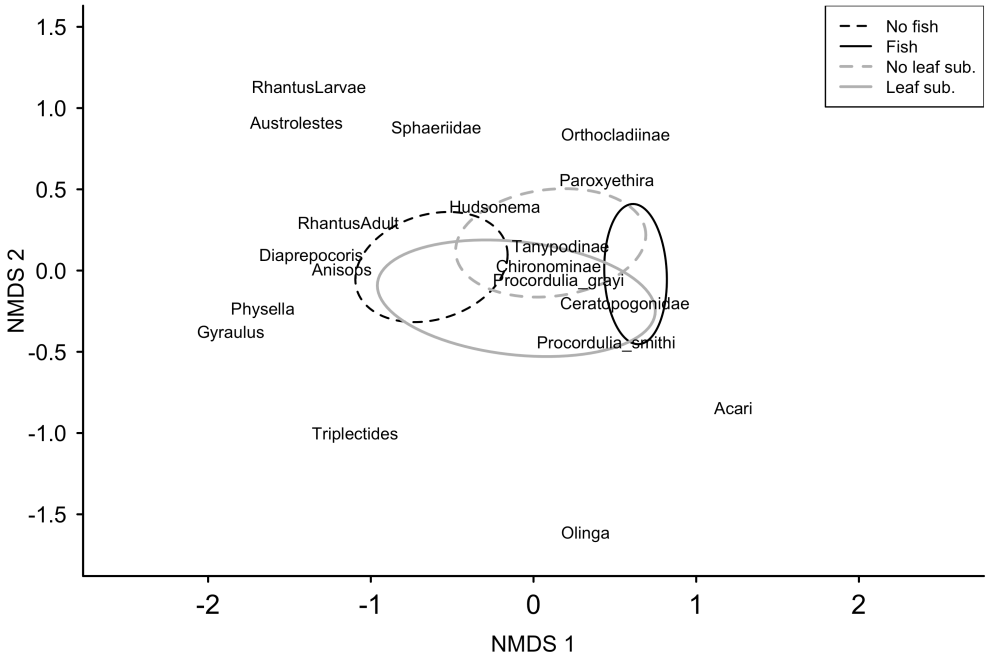


Figure 2.4: Non-metric multidimensional scaling (NMDS) ordination on invertebrate community composition for all experimental tanks, with ellipses indicating significant treatment effects of fish presence and leaf subsidy presence on community composition.

DISCUSSION

The input of cross-ecosystem subsidies can have bottom-up effects on food webs by increasing consumer abundance above what *in situ* productivity would otherwise support (Polis et al. 1997). However, variation in the trophic level at which a subsidy is consumed can lead to a multitude of food-web consequences, such as increased consumer biomass (Marczak and Richardson 2008, Dreyer et al. 2012), altered community composition (Wallace et al. 1997), and increased connections between multiple food-web compartments (Cottingham and Narayan 2013). My aim here was to examine how subsidies to predators (terrestrial insects) and subsidies to primary consumers (terrestrial leaves) influenced food-web structure. I found that the trophic level of subsidy entry fundamentally altered food-web composition and biomass, but consequences for top-down or bottom-up interactions depended on other food-web conditions, especially predator presence. Below I outline potential effects of subsidies entering at different trophic levels on top-down and bottom-up food-web interactions and how those interactions could be altered by timing of resources and presence of higher-order consumers.

Implications of resources to top-predator biomass

Insect subsidies had large, positive effects on adult fish biomass, which was not surprising because the insect subsidies were intended as a direct resource subsidy to fish. Terrestrial arthropod subsidies to fish can alter fish habitat selection (Kawaguchi et al. 2003) and multiple food-web interactions (Nakano et al. 1999, Nakano and Murakami 2001, Baxter et al. 2004). However, a comprehensive review by Baxter et al. (2005) pointed to a lack of studies investigating the effects of terrestrial arthropod subsidies on fish production, although in recent years, Sato et al. (2011) observed that fish biomass increased during seasons with highest input of terrestrial grasshoppers (Sato et al. 2011). In this study, not only did I find that adult fish biomass was increased with insect subsidies, but the production of YOY fish biomass also increased with leaf as well as insect subsidies. This indicates that subsidies increased biomass production of adult fish and their reproductive success indicated through an increase in offspring biomass.

Increased biomass of top-predator populations has the potential to increase the strength of top-down interactions. Short-term, within-generation increases to terrestrial arthropod input to freshwater systems can weaken top-down control of *in situ* prey because fish behaviourally switch from *in situ* prey to insect subsidies (Nakano et al. 1999, Baxter et al. 2004). However, predictions from models encompassing thousands of generations indicate

that long-term increases in resources to top predators will increase their biomass over multiple generations, increasing the strength of top-down control of their prey resources (Huxel and McCann 1998, Huxel et al. 2002, Leroux and Loreau 2008). Therefore the increased adult fish mass with insect subsidies that I observed could have led to strong-top down effects on lower trophic levels, which would have been indicated by a fish by insect subsidy interaction affecting primary consumers. However, the doubling of fish biomass with insect subsidies had no detectable influence on top-down control by fish on multiple metrics of primary consumer community structure.

A potential explanation for the lack of increased top-down control could be that the increased metabolic demands of higher fish biomass in insect treatments was maintained by the constant input of cross-ecosystem subsidies, meaning fish never had to increase their consumption of *in situ* prey during periods of low subsidy input. If insect subsidies were available periodically or seasonally, rather than as a constant resource, the inflated fish biomass might have increased top-down regulation during periods where subsidy input was low. For example, in temperate zones, terrestrial insect subsidies occur in pulses that peak in summer and autumn, with increased consumption of *in situ* prey probably occurring in periods between pulses (Edwards and Huryn 1995, Kawaguchi and Nakano 2001, Nakano and Murakami 2001). Moreover, consumer-resource models with pulsed subsidies generated fluctuations of *in situ* prey biomass due to increased predation during periods of low subsidies, whereas those fluctuations were not present in models with constant subsidy input (Leroux and Loreau 2012). Thus, it seems likely that the results from my experiment are most relevant to ecosystems that receive constant subsidy input through time. Furthermore, increased top-down food-web regulation, as a result of terrestrial arthropod subsidies increasing fish biomass, may only occur in systems that experience a disruption to subsidy input; either through natural periodicity or a human disturbance to the cross-ecosystem linkage.

Bottom-up effects on intermediate predators

Leaf and insect subsidies both increased biomass of the intermediate predators, the YOY fish and predatory invertebrates, providing evidence that both types of subsidies had bottom-up effects that propagated up the foods web to higher trophic levels. My observations suggest that gape limitation in YOY fish prevented their consumption of insect subsidies. Therefore the increase in their biomass from insect and leaf subsidies was probably due to an increase in carbon input to basal trophic levels that increased resource availability to primary

and secondary consumers. Past studies provide support for this hypothesis: Carpenter et al. (2005) observed positive responses of YOY fish biomass and their zooplankton prey to whole-lake additions of dissolved organic carbon, and Tanentzap et al. (2014) found similar responses in lake fish associated with a gradient of terrestrial detritus input from differentially forested watersheds. In my study, the increase in predatory invertebrate biomass in subsidy treatments could have been due to either increased consumption of primary consumers fed by leaf and detrital insect subsidies, or direct feeding by predatory insects on the insect subsidies themselves. For example, Wallace et al. (1997) found that predatory invertebrate abundance increased with terrestrial leaf subsidies and Townsend and Hildrew (1979) found the diet of a predatory stream invertebrate frequently included terrestrial arthropods. It seems likely that both of these mechanisms were operating in my experiment. In summary, although leaf subsidies did not have bottom-up effects on adult fish mass, both leaf and insect subsidies decreased bottom-up limitation, by increasing terrestrial carbon availability, at least to intermediate predators and their prey. Thus changes to the rate of input and type of basal resource subsidies may have widespread implications for the production of trophic levels throughout food webs.

Altered community structure

Leaf and insect subsidies increased predatory invertebrate biomass, but interestingly, had no effect on isotopic niche size (an indicator of the feeding interactions and taxa composition in a food web) of the predatory invertebrate guild. This is likely because the increase of predatory invertebrate biomass was mostly due to increases of *Procordulia* dragonfly larvae. Leaf and insect subsidies could have shifted the isotopic niche of *Procordulia* without affecting the niche size of the whole predatory invertebrate guild. Predatory invertebrate isotope niche size, was however, decreased by fish presence. The decreased niche size was likely due to loss of certain taxa in the presence fish, such as *Rhantus*, *Antiporus*, and *Liodessus* adult beetles that had relatively negative δ13C. Therefore, changes in isotopic niche size of predatory invertebrates were most likely due to changes in taxa composition, opposed to altered food resources from subsidies.

Insect subsidies decreased the isotope niche size of primary consumers and did not affect community composition of primary consumers, however, fishless tanks receiving insect subsidies had the highest taxa diversity of all experimental food webs. Positive relationships between resource supply and diversity have been observed in past studies (Cardinale et al. 2009), so it appears likely that resource heterogeneity and/or total

availability of detrital resources to primary consumers with the addition of insect subsidies could promote increases in diversity. By entering the detrital pathway, subsidies normally destined to be fed upon by predators, can instead increase high quality resources to primary consumers, therefore increasing resource heterogeneity (Nowlin et al. 2007, Hoekman et al. 2011). In addition, increased resource quality can mediate antagonistic interactions and competitive exclusion, resulting in higher diversity of co-existing taxa. For example, Wissinger et al. (2004) found that the addition of high protein resources can reduce competitive exclusion in guilds of closely-related pond caddisflies by mediating interspecific competition and intraguild predation. Therefore, the insect subsidies may have increased resource diversity and decreased competition, leading to an increase in primary consumer diversity.

In contrast to insect subsidy effects, leaf subsidies increased primary consumer isotopic niche size and altered invertebrate community composition. The altered isotopic niche space with leaf subsidies was mainly due to an increase in δ15N. While increased δ15N can indicate a shift in trophic level at which an organism is feeding (Post 2002), the elevated δ15N signatures of leaf subsidies compared to *in situ* algae, in my experiment suggests that increased consumption of willow leaf subsidies by primary consumers is a more likely explanation of increased primary consumer δ15N with leaf addition. Primary consumers most associated with leaf subsidies were *Physa* and *Gyraulus* snails as well as *Triplectides* and *Olinga* caddisfly larvae; all of which are generalist feeders that readily consume particulate detritus either directly or as a result of grazing leaf biofilms (Stark 1981, Winterbourn 2000). In addition to responding to increased detrital resources, these taxa may have also benefited from the habitat structure provided by leaf detritus, as seen in the response of generalist taxa to detritus inputs in other systems (Moore et al. 2004, Hagen et al. 2012a). Thus, by potentially providing alternate food and structural resources, leaf subsidies led to compositional changes in primary consumers.

The altered community composition and niche size as a result of subsidy inputs has the potential to influence the presence and coupling (through higher-level predators) of fast and slow food-web compartments that underlie food-web stability. Rooney et al. (2006) predicted that detrital resources in food webs generates compartments of slow energy flow that, when consumed by a shared predator, dampen the consumer and resource fluctuations in autochthonous channels with rapid energy flow. Moreover, Rooney and McCann (2012) found that increased food-web stability was associated with an increase in diversity within detrital food-web compartments. In my tanks, leaf subsidies may have

stabilised food webs by creating multiple food-web compartments (*in situ* resource and subsidy resource compartments) that increased resource diversity to higher-level consumers. However, with the presence of higher-level consumers (adult fish) that could potentially couple the compartments, leaf subsidies did not increase isotopic niche size of primary consumers. This could be largely because fish either extirpated or prevented the establishment of primary consumers that readily consume leaf detritus (i.e. *Physa* and *Gyraulus* snails and *Triplectides* and *Olinga* caddis). This suggests that the predators that have the ability to couple food-web compartments can also reduce the potential for these compartments to form within food webs, potentially decreasing stabilising effects of leaf subsidies (see Chapter Three for coefficient of variation analysis). Thus, strong top-down interactions may decrease the bottom-up effects of subsidies to primary consumers and alter the establishment of structures such as fast and slow food web compartments that stabilise food webs.

Conclusions

Model predictions and past empirical research show that cross-ecosystem subsidies have the potential to increase consumer biomass and alter whole-food web interactions through changes in bottom-up regulation. Although the anticipated bottom-up propagation of resources from consumers to higher-level predators did occur in my study, subsidy effects on food-web interactions were altered by properties of the terrestrial subsidy (insects versus leaves) and the recipient aquatic food webs (i.e.. those with and without fish). Top-predators can utilize the increase in resources of terrestrial insect subsidies, but whether that results in top-down cascades to lower trophic levels depends on subsidy timing and frequency. Subsidies to primary consumers, by comparison, have the potential to increase food-web compartments, but strong top-down interactions reduce those bottom-up effects. These results point to subsidy trophic level determining bottom-up regulation in food webs, but overriding factors, such as subsidy frequency from donor ecosystems and predator presence in recipient ecosystems, may reduce subsidy impact on food-web interactions. Moreover, these outcomes suggest changes in terrestrial and aquatic food webs that alter the nature and receipt of subsidies have the potential to fundamentally alter the consequences of cross-ecosystem exchanges.

Photo: Angus McIntosh

Chapter Three: CAUSES OF VARIABILITY AND STRENGTH OF TROPHIC CASCADES REVEALED BY RESOURCE SUBSIDIES TO MULTIPLE TROPHIC LEVELS

INTRODUCTION

Trophic cascades are a common, natural phenomenon involving indirect top-down influences of predators on primary producers (Hairston et al. 1960). Since the introduction of this concept by Hairston, Smith, and Slobodkin (HSS) (1960), whether food webs are regulated by top-down or bottom-up forces has been hotly debated (Hunter and Price 1992, Power 1992). However, top-down and bottom-up influences on food-web interactions in natural systems act in concert (Figure 3.1), and recent studies have focussed on what determines their relative importance and strength (Leibold et al. 1997, Borer et al. 2005, Heath et al. 2014). That top-down regulation not only affects the biomass or density of a trophic level, but can also cause variability in that biomass, further complicates this issue. Variation in biomass of a trophic level can decrease with the presence of strong top-down interactions, such as predation, (O'Gorman and Emmerson 2009), and increase with bottom-up resources (Rosenzweig 1971). Therefore, it is important to consider how the combination of altering bottom-up food-limitation and top-down consumer regulation alter the link between trophic cascades and biomass variation at multiple trophic levels.

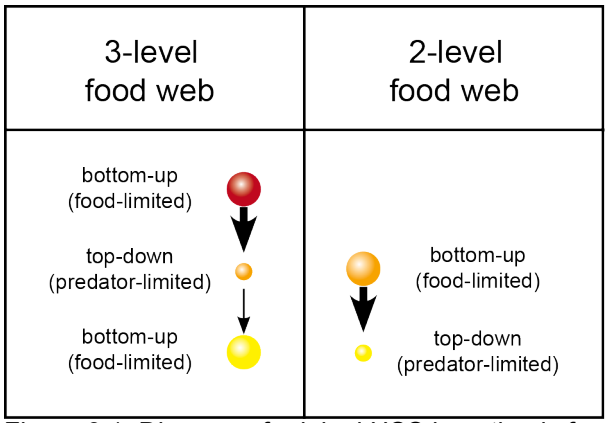


Figure 3.1: Diagram of original HSS hypothesis for top-down versus bottom-up food-web regulation. Spheres represent different trophic levels and arrows represent interactions between trophic levels, with the size of spheres indicating the prevalence of that trophic level within an ecosystem, and the size of arrows representing the strength of interactions. Red spheres, predators; orange spheres, primary consumers; and yellow spheres, primary producers.

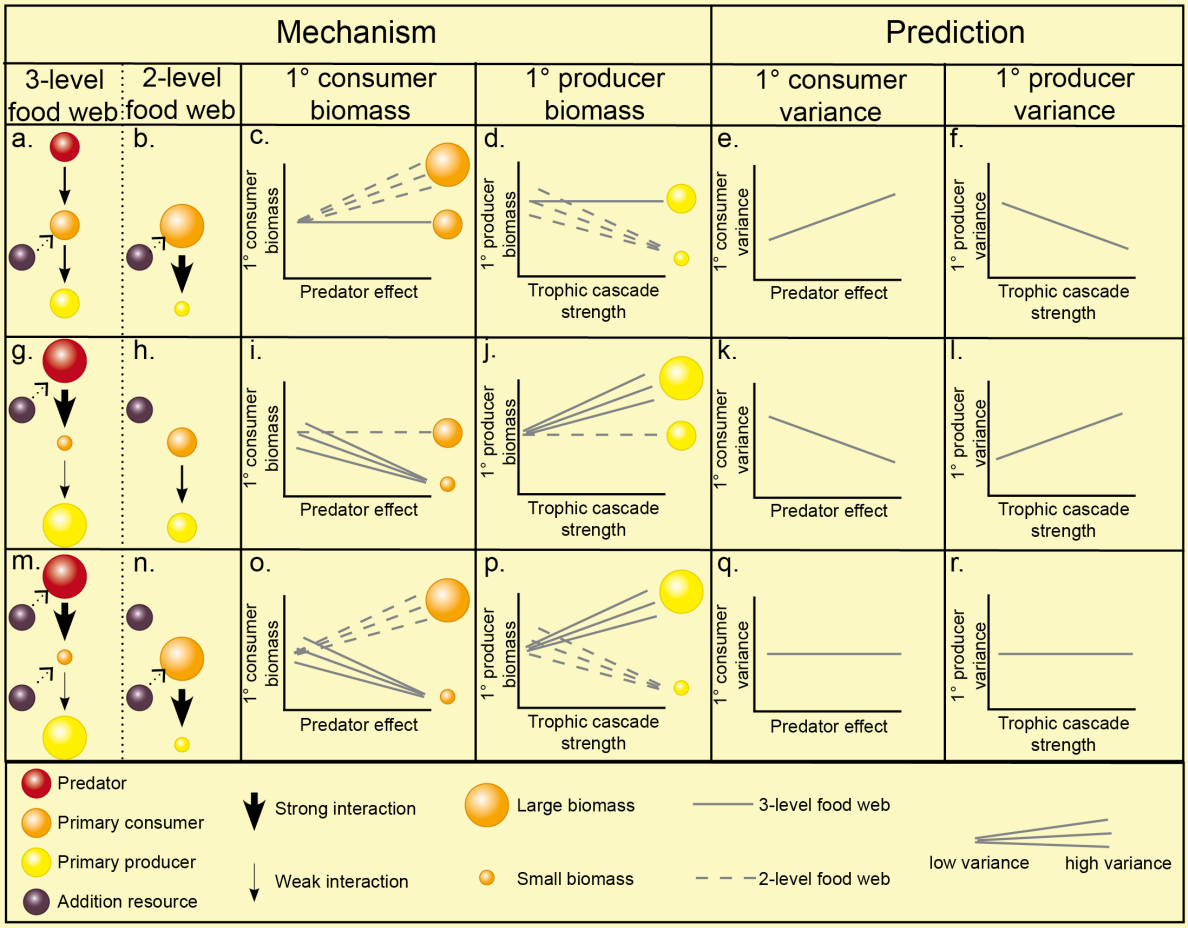
Trophic cascades are driven by properties of primary consumer and predator guilds, such as bottom-up resource-limitation, that alters top-down regulation of primary production (Borer et al. 2005). For instance, when primary consumers are food-limited their populations are responsive to increases in resources (Leibold 1989, Cebrian 1999), altering primary consumer and primary producer biomass. Predators, on the other hand, restrict the ability of primary

consumers to increase in response to increasing resources. Nevertheless, if predators are resource-limited themselves and numerically increase in biomass with added resources (Polis 1999), the inflated predator biomass can create strong top-down regulation of prey. When both of these forces act in concert, top-down and bottom-up regulation of primary consumers and primary producers leads to strong trophic cascades.

Multiple factors can alter bottom-up resource availability to primary consumers and predators (Ostfeld and Keesing 2000). For example, seed masting years in *Nothofagus* forests can create a flux of resources that cascades up to terrestrial primary consumers, eventually increasing top-predator numbers (O'Donnell and Phillipson 1996). In contrast to the influences of these *in situ* resources, cross-ecosystem subsidies can provide a unique type of resource to multiple food-web trophic levels because subsidies are donor-controlled and do not contribute to *in situ* feedback loops between consumers and prey (Polis et al. 1997). By disconnecting the feedbacks between predators and *in situ* prey, cross-ecosystem resources can potentially have large influences on bottom-up and top-down food-web regulation by maintaining consumers at higher biomass than could be supported by *in situ* resources (Henschel et al. 2001, Baxter et al. 2004, Piovia-Scott et al. 2011).

Importantly, cross-ecosystem resources that are consumed by predators may also differ mechanistically in their effects on food webs, compared to those consumed by primary consumers. Experimental decreases of subsidies to predators (e.g. reductions in terrestrial arthropods to stream fish) can cause predators to switch from feeding on subsidies to feeding on *in situ* primary consumers, increasing trophic cascade strength as subsidies decrease (Nakano et al. 1999). In contrast, increases in subsidies of primary producers (e.g. subsidies of terrestrial leaves to stream detritivores or seaweed to detritivores on rocky shores) may increase trophic cascade strength by supporting high predator numbers through increases in detritvores available as prey (Polis and Hurd 1995, Nyström et al. 2003). However, the timescales of both of these mechanisms likely varies, potentially producing a variety of effects. Trophic cascades due to short-term, behavioural changes in predatory prey-selection, may dissipate as more long-term, numerical food-web responses, driven by changes in abundance, take place. Nevertheless, theory suggests that increasing subsidies to any trophic level, given enough time, will increase biomass up the food web, eventually leading to higher predator biomass, and therefore increased top-down effects on primary producers (Leroux and Loreau 2008).

Box 3.1



Strong top-down interactions can decrease variability in biomass/abundance within trophic level (Hillebrand 2008, O'Gorman and Emmerson 2009), while bottom-up regulation can increase variability in biomass (Rosenzweig 1971). Therefore, increasing bottom-up resources to both primary consumer and top-predator trophic levels could affect primary consumer and primary producer variability by altering top-down and bottom-up dynamics at those trophic levels. There are three mechanisms that can increase trophic cascade strength with added resources that lead to different predictions regarding biomass variability at primary consumer and primary producer trophic levels:

Increases in resources to primary consumers in 2-level food webs (b) would lead to an increase in primary consumer biomass (c, dashed lines). However, in 3-level food webs (a), predation would decrease the response of primary consumers to added resources (c, solid line). Thus increased resources in a 2-level food web would lead to increased consumption, and therefore decreased biomass, of primary producers (d, dashed lines), with no change in primary producers in 3-level food webs (d, solid line). Thus trophic cascades, in this case, are driven by decreases in producer biomass in the absence of predators. Because these top-down effects of added resources are driven by increased resources to primary consumers and their increased consumption on primary producers, I predict that variation of primary consumer biomass will increase with predator effect (e), while variation of primary producer biomass will decrease with trophic cascade strength (f).

If resources to predators increase in 3-level food webs (g), predator biomass will likely increase leading to increased predation and a decrease in primary consumer biomass (i, solid lines). However in 2-level food webs (h), the absence of predators mean resources are not consumed, and there would be no change in primary consumer biomass (i, dashed line). Decreased primary consumer biomass would release primary producers from consumption in 3-tier food webs leading to an increase in primary consumer biomass (j, solid lines). Primary producer biomass would remain constant in 2-level food webs as consumption by primary consumers does not change (j, dashed line). Because these top-down effects of added resources are driven by increased predation on primary consumers and primary producers being released from consumption, I predict that variation of primary consumer biomass decrease with predator effect (k), while variation of primary producer biomass will increase with trophic cascade strength (l).

Finally, the combined effects of increases in resources to both primary consumers and top-predators, will lead to increases in primary consumer biomass in 2-level food webs (n&o, dashed lines) and decreases in 3-level food webs (m&o, solid line). Similarly, effects on primary producer biomass will be a combination of the added resources, with an increase in 3-level food webs (m&p) and decrease in biomass in 2-level food webs (n&p). With both top-down and bottom-up regulation acting in concert on both primary consumer and primary producer biomass, I predict that there will be no change in variability of biomass with increasing trophic cascade strength (q&r).

Using cross-ecosystem subsidies to increase resources to predators and primary consumers in experimental pond mesocosms, I empirically investigated how altering bottom-up regulation within food webs affected 1) top-down trophic cascades and 2) variability within primary consumer and primary producer trophic levels. I hypothesized that increasing subsidies to primary consumers would create strong trophic cascades, which at first occurred because of primary consumers prey-switching from primary producers to subsidies, but were later caused by numerical increases in primary consumer biomass due to reduced food-limitation (Box 3.1a-d). Moreover, because subsidies are donor-controlled and do not influence *in situ* prey-consumer feedback loops, I predicted the bottom-up increase in resources was likely to increase variation of primary consumer biomass through instances of runaway production (Box 3.1e; Rosenzweig 1971), and decrease variation in primary producer biomass through increased strength of top-down interactions (Box 3.1f; Hillebrand 2008, O'Gorman and Emmerson 2009). I also hypothesised that increasing subsidies to top-predators would create short-term negative impacts on algae due to behavioural prey-switching by predators, which later led to strong trophic-cascades as numerical increases in predator biomass increased top-down control on *in situ* primary consumers (Box 3.1g-j). This reduction in predator food-limitation may also cause decreased variation of primary consumer biomass due to increased top-down interactions (Box 3.1k; O'Gorman and Emmerson 2009) and increased variation of primary producer biomass because they are now released from predation and subject to bottom-up regulation (Box 3.1l; Rosenzweig 1971). Lastly, I hypothesized that increasing resource subsidies to both primary consumers and predators would at first reduce-top down control due to prey-switching at both predator and primary consumer trophic levels (effectively cancelling each other out), but would later lead to strong trophic cascades due to numerical increases in both predator and predator-free food webs (Box 3.1m-p). In this case, because both top-down and bottom-up forces are acting simultaneously on primary consumer and primary producer trophic levels, variation of primary consumer and primary producer biomass will likely remain unaffected (Box 3.1q&r). Importantly, using subsidies to test these hypotheses will likely create stronger bottom-up and top-down effects than altering *in situ* resources, because external resources typically offset negative feedbacks and population cycles that occur when consumer abundance tracks the density of their prey (Polis et al. 1997).

METHODS

Experimental set-up

The 18-month pond mesocosm experiment took place in the Southern Alps, South Island, New Zealand at the University of Canterbury's Cass Mountain Research Station. In January 2013, thirty-two 1,100 litre cattle tanks (mesocosms) were filled with ground water, 1 cm of gravel substrate, common pond macrophytes from the area (submergent *Myriophyllum* and emergent *Carex*), and two 10-cm diameter ceramic pots positioned on their side to provide fish habitat. Experimental tanks were inoculated with 5 l of filtered pond water, 380 ml aliquots of concentrated phytoplankton and zooplankton, and 300 ml filtered fine particulate organic matter. To ensure representation of various trophic levels and feeding groups, a range of benthic invertebrates from local ponds and lakes were added to tanks in natural densities based on exploratory surveys (predatory invertebrates: 10 *Procordulia* dragonflies and 10 *Xanthocnemis* damselfies; primary consumers: ~200 *Potamopygrus* snails, ~100 Chironominae midges and 50 *Triplectides* caddisflies). In addition to those taxa, two 0.3 m^2 sweeps of benthos with a 1-mm mesh D-net from local ponds (one from a permanent pond and one from a temporary pond) were added to the tanks to increase diversity of rare taxa to mimic naturally occurring food webs. Tanks were left to be naturally colonised by terrestrial dispersal of adult stages of invertebrates from January to May 2013 before experimental treatments were added; natural colonization continued to occur throughout experiment.

The experiment had a fully crossed 2 x 2 x 2 factorial design, with the presence/absence of subsidies to primary consumers (leaves) and the presence/absence of subsidies to top-predators (beetle larvae) crossed with the presence/absence of top-predators (fish). Each treatment was replicated four times and randomised within four spatial blocks of tanks. Ten adult upland bullies (*Gobiomorphus breviceps*) (totalling 26.6 +/- 0.4 g), a common native predatory fish in New Zealand freshwater ecosystems (Staples 1975), were added as the top predator to "fish" treatment tanks on 14 May 2013. These adult fish reproduced in December 2013, and young-of-the-year (YOY) upland bullies were present from this point onward.

Four grams of air-dried riparian willow leaves (*Salix fragilis,* the most common riparian tree in the area) were added as resources to primary consumers ("leaf subsidy") every four weeks, beginning 22 May 2013. Every two weeks, beginning 22 May 2013, two

grams of live beetle larvae (*Tenebrio* sp.) were added as resources to predatory fish ("insect subsidy"). Beetle larvae were used so I could easily control subsidy additions.

Food-web sampling

Six unglazed terracotta saucers (0.005 m^2) were placed in the tanks as artificial substrate for measuring the top-down effects of fish on benthic algae biomass. Over the course of the experiment, one artificial substrate was sampled on each of six dates (18 July 2013, 8 November 2013, 23 January 2014, 11 April 2014, 17 July 2014, and 3 November 2014). Substrates were removed from tanks and stored in the dark until transported to the laboratory where they were frozen at -22°C until processed. To lyse the algal cells and release photosynthetic pigments, these substrates were submerged in 50 mL of 90 % ethanol buffered with magnesium carbonate, placed in a 78°C water bath for 5 minutes, and stored at 5°C. After 12 to 18 h, a 5-mL aliquot was analysed for chlorophyll-*a* (chl *a*) concentration with a Turner Designs Trilogy Laboratory Flurometer (model #7200), treated with 0.15 mL of 0.1 normal hydrochloric acid, and then re-analysed on the fluorometer to account for phaeophytin. Raw fluorescence units were converted to μg/cm^2 by subtracting fluorescence (post acid) from fluorescence (pre acid) and using a standard fluorescence curve against a chl *a* standard (adapted from Arar and Collins 1997).

Five 0.04-m^2 mesh sampling baskets containing cobble and fabric leaves were placed in each tank for sampling the top-down effect of fish on benthic invertebrates. On each of the four sampling dates (18 July 2013, 8 November 2013, 23 January 2014, and 3 November 2014), one basket was removed from each tank and all invertebrates were separated from the basket and preserved in ethanol for later identification. Baskets were then returned to the tanks to keep structural habitat consistent through time. In the laboratory, all invertebrates >0.5 mm in length were identified to lowest taxonomic unit, usually genus or species, using Winterbourn (2006), and categorized into primary consumers and predatory invertebrates (following Greig 2008). They were then photographed using a Leica DFC450 microscope camera. Length measurements were taken by uploading the digital photographs to Adobe Acrobat Pro and measuring the invertebrates using the 'Measuring Distance' tool. Dry weight was calculated using length-weight regressions (Towers et al. 1994, Nyström and Pérez 1998, Benke et al. 1999, Baumgärtner and Rothhaupt 2003, Stoffels et al. 2003, Greig 2008). Invertebrates were categorized as predatory invertebrates or primary consumers. Primary consumers were categorised as a general feeding group, rather than specifically separating

them as algivores or detritivores, because most species within the communities were facultative consumers of both food resources (Stark 1981).

In December 2013, adult fish that were placed in the tanks at the beginning of the experiment reproduced, and young-of-year (YOY) upland bullies were present from this point onward. At the end of the experiment, after 532 days, fish were removed from the tanks, and adult and YOY fish were counted and weighed separately.

Statistical analysis

Primary consumer dry mass and algal biomass (chl *a*) were modelled using linear mixed effects models with insect subsidies, leaf subsidies, fish presence, and date, and their interactions as fixed categorical factors and tank as a random factor. Correcting for possible autocorrelation of tank through time by using a first-order autoregressive model did not improve model fit, so this term was not retained in final models (Crawley 2012). Post-hoc mixed-effects models for primary consumer dry mass and algal biomass were performed for individual dates with insect subsidies, leaf subsidies, and fish presence, and their interactions as fixed categorical factors and block as a random factor. All response variables were $\log_{(e)}$-transformed to meet assumptions of normality. All linear mixed effects models were run using 'nlme' package (Pinheiro et al. 2015) in R (R Core Team 2014).

Interaction strengths were expressed as effect sizes (Shurin et al. 2002), whereby top-down fish effects on algal biomass (i.e. trophic cascade strength) and primary consumer dry mass were calculated with the $\log_e$ ratio effect size:

$$\text{fish effect size} = \log_e(\text{response}_{\text{fish}}/\text{response}_{\text{nofish}});$$

where $\text{response}_{\text{fish}}$ was either primary consumer dry mass or algal biomass from tanks with fish and $\text{response}_{\text{nofish}}$ was either primary consumer dry mass or algal biomass from tanks without fish. To evaluate effect sizes using the whole range of replicate fish treatment and fishless treatment tanks, I used bootstrapping methods to calculate means and 95% confidence intervals. Computer generated random pairings of individual replicate fish tanks with individual replicate fishless tanks were repeated 10,000 times with replacement, with pairings of fish and fishless tanks nested within subsidy treatment, which was nested within date. Means and 95% confidence intervals were then calculated from the 10,000 randomisations, giving the best possible measure of fish effect size.

To investigate the relationship between primary consumer dry weight in fish and fishless treatment tanks and the bootstrapped fish effect on primary consumers, an analysis of covariance (ANCOVA) was run with fish effect on primary consumers and categorical fish

presence as independent variables, and $\log_e$-transformed primary consumer dry weight as dependent variable. If the interaction between fish effect and fish presence was signficant ($p < 0.05$), separate post-hoc regressions were run for fish and fishless treatments. The same analysis was run on the relationship between algal biomass in fish and fishless treatment tanks and the bootstrapped trophic cascade strength using an ANCOVA with independent variables being trophic cascade strength and categorical fish presence, and dependent variables $\log_e$-transformed algal biomass. Post-hoc regressions for fish and fishless treatments were run if the interaction between trophic cascade strength and fish presence was significant ($p < 0.05$).

Spatial patchiness and temporal variability of primary consumer dry weight and algal biomass were measured as the coefficient of variation (CV = standard deviation$_T$/mean$_T$), where T was either treatment for a given date (spatial CV) or a tank across dates (temporal CV). Analysis of variance (ANOVA) was run for $\log_e$-transformed spatial CV (sampling date used as replicate) and temporal CV (using treatment replicates) with insect subsidy, leaf subsidy, fish presence, and the interactions as predictor variables. CV was $\log_{(e)}$-transformed to meet assumptions of normality. ANCOVA was used to investigate how spatial CV (for primary consumer dry mass and algal biomass) covaried with bootstrapped fish effect (fish effect on primary consumer and trophic cascade strength) and subsidy trophic level. All analyses were completed using R (R Core Team 2014).

RESULTS

Trophic cascade strength

Trophic cascade strength naturally fluctuated during the 18-month experiment in food webs not receiving additional resource subsidies (Figure 3.2a.). After 58 days, on the first sampling date, trophic cascade effect sizes were on average strongest in food webs where top-predators were receiving additional resources through insect subsidies, but the mean of their effect diminished through time (Figure 3.2c). In food webs where only primary consumers were receiving additional resources through leaf subsidies, mean trophic cascade effect size increased through time (Figure 3.2b). This meant that by the end of the experiment, fish tanks receiving leaves had double the algal biomass of fishless tanks receiving leaves. Interestingly, when both top-predators and primary consumers were receiving additional resources, trophic cascades were consistently absent, until day 532 (Figure 3.2d). Therefore, subsidy trophic level altered trophic cascade strength, and depending on the type of subsidy, those effects changed through time.

Table 3.1: Analysis of variance models on primary consumer (a) biomass (after 58, 248, and 532 days), (b) temporal variation (temporal C.V.), and (c) spatial variation (spatial C.V.) and also on algal (d) biomass (after 58, 248, and 532 days), (e) temporal variation (temporal C.V.), and (f) spatial patchiness (spatial C.V.). Values are p-values and bold indicates significance. I = presence/absence insect subsidies; L = presence/absence leaf subsidies; F = presence/absence fish; D = date; dashes = predictors that were not included in the model.

Response variable	Predictor variables														
	I	L	F	D	IxL	IxF	LxF	IxD	LxD	FxD	IxLxF	IxLxD	IxFxD	LxFxD	IxLxFxD
Primary Consumers															
(a)Biomass*	0.226	0.069	**<0.001**	**0.018**	0.772	0.707	0.674	0.234	0.272	0.146	0.732	0.527	0.645	0.594	0.705
Day 58	0.172	0.098	**0.022**	-	0.3608	0.306	0.941	-	-	-	0.3117	-	-	-	-
Day 248	0.872	0.455	**<0.001**	-	0.854	0.913	0.133	-	-	-	0.622	-	-	-	-
Day 532	0.955	0.247	**<0.001**	-	0.291	0.819	0.528	-	-	-	0.502	-	-	-	-
(b)Temporal variation*	0.901	0.126	0.452	-	**0.022**	0.401	0.583	-	-	-	0.979	-	-	-	-
(c)Spatial patchiness*	**0.019**	**0.024**	0.697	-	0.244	0.138	**0.004**	-	-	-	0.086	-	-	-	-
Algae															
(d)Biomass*	**0.021**	0.288	**<0.001**	**<0.001**	0.366	0.375	0.903	0.484	0.948	**0.007**	0.184	0.357	0.639	0.162	0.955
Day 58	0.132	0.524	0.145	-	0.252	0.075	0.129	-	-	-	0.493	-	-	-	-
Day 248	0.694	0.489	0.748	-	0.551	0.838	0.476	-	-	-	**0.048**	-	-	-	-
Day 532	0.152	0.499	**0.005**	-	0.203	0.676	0.116	-	-	-	0.997	-	-	-	-
(e)Temporal variation*	0.148	0.879	**0.001**	-	0.166	0.721	0.557	-	-	-	0.729	-	-	-	-
(f)Spatial patchiness*	0.212	0.591	0.579	-	0.818	0.975	0.841	-	-	-	0.742	-	-	-	-

* Indicates $\log_e$-transformed response variable

Primary consumer biomass changed through time and there was almost a significant decrease in their biomass with leaf subsidies (leaf main effect, p=0.069; Table 3.1a; Figure 3.3a-c). The most drastic change in primary consumer biomass, however, was caused by the presence of fish; in extreme instances primary consumer biomass was 3.8 times lower than that of fishless food webs (fish main effect, p<0.001; Table 3.1a; Figure 3.3a-c). This was reflected in large negative fish effect sizes on primary consumers across all dates and resource addition treatments (Figure 3.3g-i), except day 58 where leaf subsides decreased the fish effect (Table 3.1a).

Post-hoc ANOVAs with leaf subsidies, insect subsidies, and their interactions as independent variables, on fish and fishless tanks for each date revealed that added resources did alter primary consumer dry mass in fishless tanks, but the effects varied greatly over time. At day 58, insect subsidies increased primary consumer biomass, but only in tanks not receiving leaf subsidies (insect x leaf, p=0.045). The significant positive effect of insect subsidies in fishless tanks decreased in days 248 and 532 (Figure 3.3 b&c). Thus, subsidies did alter primary consumer biomass, but the top-down fish effect on primary consumer biomass was stronger.

Insect subsidies, as well as fish presence, significantly increased algal biomass (insect main effect, p=0.021; Table 3.1d; Figure 3.3d-f; fish main, effect p<0.001; Table 3.1d; Figure 3.3d-f). Algal biomass significantly changed through time, quadrupling after 248 days (sampling date main effect p<0.001; Table 3.1d; Figure 3.3d-f). Also, on day 248 there was a

significant insect x leaf x fish interaction where fish decreased algal biomass, but only with the input of both insect and leaf subsidies (insect x leaf x fish interaction, p=0.048; Table 3.1d; Figure 3.3e).

Table 3.2: Analysis of covariance models on log_e-transformed primary consumer (a) biomass and (b) spatial patchiness (spatial C.V.), and on log_e-transformed algal (c) biomass and (d) spatial patchiness (spatial C.V.). Values are p-values and bold indicates significance. FE = fish effect size (primary consumers) or trophic cascade effect size (algae); I = presence/absence insect subsidies; L = presence/absence leaf subsidies; F = presence absence fish; dashes = predictors that were not included in the model.

Response variable	**Predictor variables**								
	FE	**I**	**L**	**F**	**FE x I**	**FE x L**	**FE x F**	**IxL**	**FExIxL**
Primary Consumers									
(a) Biomass*	**0.042**	-	-	**<0.001**	-	-	**<0.001**	-	-
No fish	**<0.001**	-	**-**	**-**	**-**	**-**	**-**	-	-
Fish	0.197	-	-	**-**	**-**	**-**	**-**	-	-
(b) Spatial variability*	0.967	**0.037**	**0.046**	-	0.725	**0.015**	-	0.505	0.801
No leaf	0.069	**-**	**-**	-	-	-	-	**-**	-
Leaf	**0.036**	-	-	**-**	**-**	**-**	**-**	-	-
Algae									
(c) Biomass*	0.433	-	-	**<0.001**	-	**-**	**<0.001**	-	-
No fish	**<0.001**	-	-	-	-	-	-	-	-
Fish	**0.015**	-	-	-	-	-	-	-	**-**
(d) Spatial variability*	**<0.001**	**0.025**	0.440	-	0.312	0.477	-	0.131	0.115

* Indicates log_e-transformed response variable

ANCOVA indicated that increasing fish effect sizes on primary consumers were due to primary consumer biomass increasing in fishless tanks, but not changing in fish tanks (ANCOVA fish effect size x fish presence, p<0.001; fishless tank fish effect main effect, p<0.001; Figure 3.4a; Table 3.2a). However, increasing trophic cascade strength (fish effect on algal biomass) was due to both increased algal biomass with fish and decreased algal biomass without fish (fish effect size x fish presence, p<0.001; fishless tank fish effect main effect, p<0.001; fish tank fish effect main effect p=0.015; Table 3.2c; Figure 3.4b). Thus, increasing fish effects on primary consumer biomass were due to changes in fishless tanks, but increasing trophic cascade strength on primary producer biomass was due to changes in fish and fishless tanks.

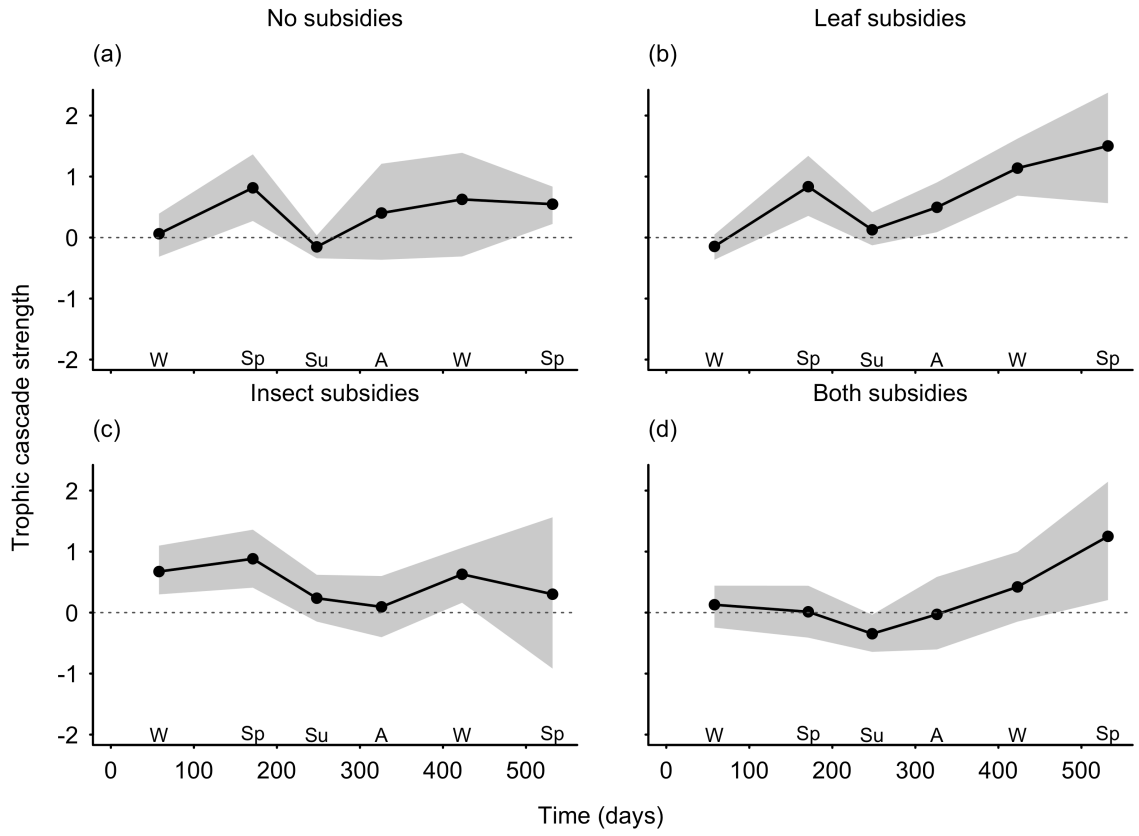


Figure 3.2: Bootstrapped means and 95% confidence intervals (grey shading) for trophic cascade effect size at six sampling dates spanning 532 days in (a) no subsidy, (b) leaf subsidy, (c) insect subsidy, and (d) both insect and leaf subsidy treatments. Significant trophic cascades are indicated by grey shading not overlapping zero. The seasons samplings took place is indicated along the x-axis by W (winter), Sp (spring), Su (summer), and A (autumn). Trophic cascade strengths were calculated as effect sizes using the $\log_e$-ratio of primary producers in the presence and absence of top predators (see methods for more details).

Temporal and spatial variability

Temporal variability of primary consumer biomass with insect subsidies was dampened with simultaneous input of insect and leaf subsidies (insect x leaf, p=0.022; Table 3.1b; Figure 3.5a). Spatial variability of primary consumer biomass was decreased when either insect or leaf subsidies were added (insect main effect, p=0.019; leaf main effect, p=0.024; Table 3.1c; Figure 3.5c). Fish also decreased spatial variability of primary consumer biomass, but only with the input of leaf subsidies (leaf x fish, p=0.004; Table 3.1c; Figure 3.5c). Furthermore, ANCOVA revealed the relationship between fish effect sizes on primary consumer biomass and spatial variability of primary consumer biomass significantly decreased with leaf subsidy input (fish effect size x leaf, p=0.015; fish effect main effect (leaf treatments), p=0.036; Table 3.2b; Figure 3.5e). In comparison, spatial variability in primary consumer biomass decreased with insect subsidies irrespective of fish effect size (insect main effect, p=0.037 and no fish effect size interaction; Table 3.2b; Figure 3.5e). Overall, leaf

subsidies tended to decrease variability of primary consumer biomass, while insect subsidies had variable influence depending on whether the response was temporal or spatial variation.

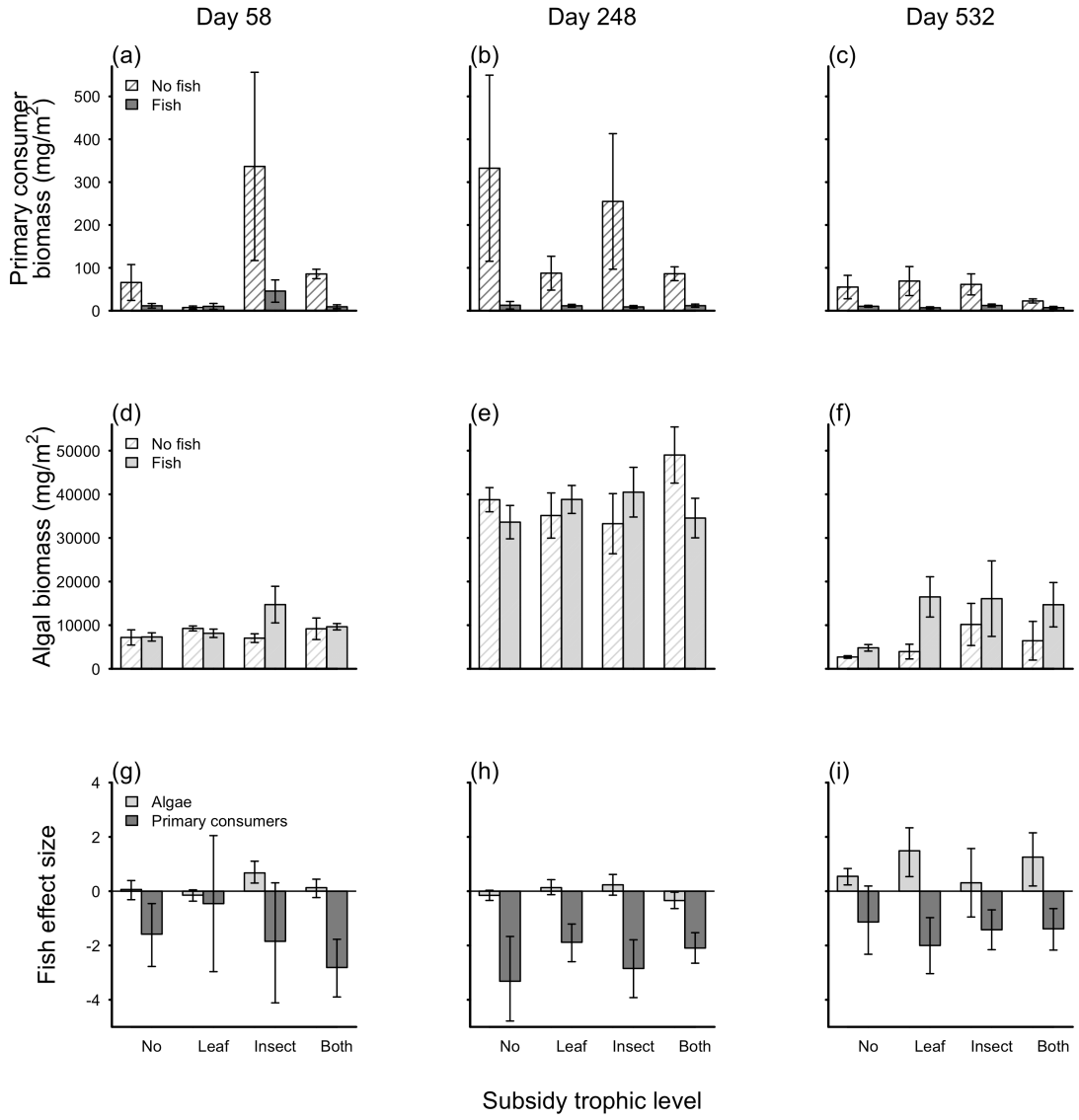


Figure 3.3: Mean (+/- standard error) in fish (solid bars) and no fish (hashed bars) tanks for primary consumer dry weight (mg/m^2) after (a) 58, (b) 248, and (c) 532 days; algal biomass (mg/m^2) after (d) 58, (e) 248, and (f) 532 days; and bootstrapped means and 95% confidence intervals of fish effect sizes (i.e. trophic cascade strength) for algal biomass (light grey bars) and primary consumer biomass (dark grey bars) after (g) 58, (h) 248, and (i) 532 days.

Temporal variability in algal biomass was decreased with fish presence (fish main effect, p=0.001; Table 3.1e; Figure 3.5b). However, there were no significant effects of insect subsidies, leaf subsidies, and fish on spatial variability of algal biomass (Table 3.1f; Figure 3.5d). Nevertheless, ANCOVA indicates trophic cascade strength was significantly positively related with spatial variability of algal biomass (fish effect size main effect, p<0.001; Table 3.2d; Figure 3.5f), with insect subsidies increasing spatial variability of algal biomass for any

given level of fish effect size (insect main effect, p=0.025; Table 3.2d; Figure 3.5f). Therefore, fish decreased temporal variation of primary producer biomass, yet spatial variation increased with top-down fish effect (trophic cascade strength).

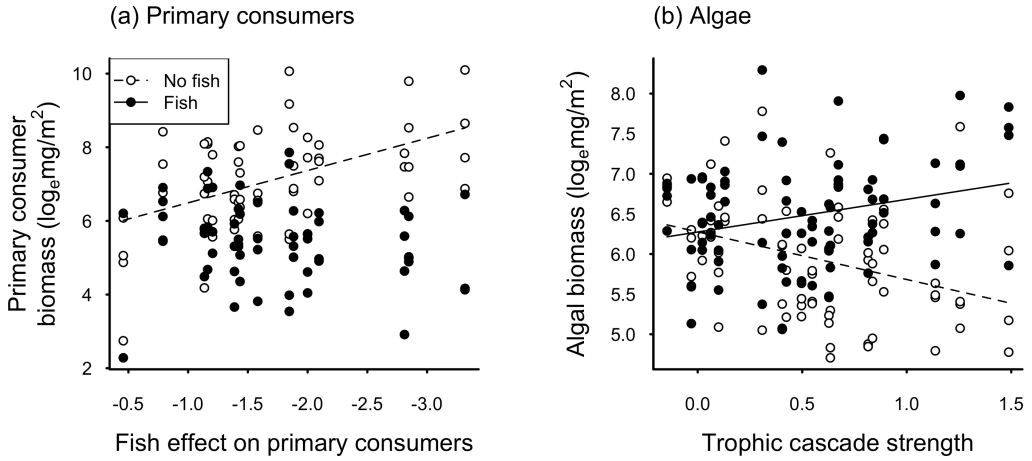


Figure 3.4 ANCOVAs were performed to examine the causes of increased predator effect size and trophic cascade effect size associated with influences on primary consumer and algal biomass. The primary consumer and primary producer biomasses from fish (black points and solid lines) and fishless (open points and dashed lines) tanks that went into the calculation of fish effect size on (a) primary consumers and (b) trophic cascade strength, respectively. Fish effect increases were due to an increase in primary consumer biomass (mg/m^2) in fishless tanks and no change in primary consumer biomass (mg/m^2) in fish tanks. Trophic cascade increases were due to a decrease in algal biomass in fishless tanks and a increase in algal biomass in fish tanks. Algal biomass from sampling date 248 was not included in the graph, because that sampling date was an extreme outlier.

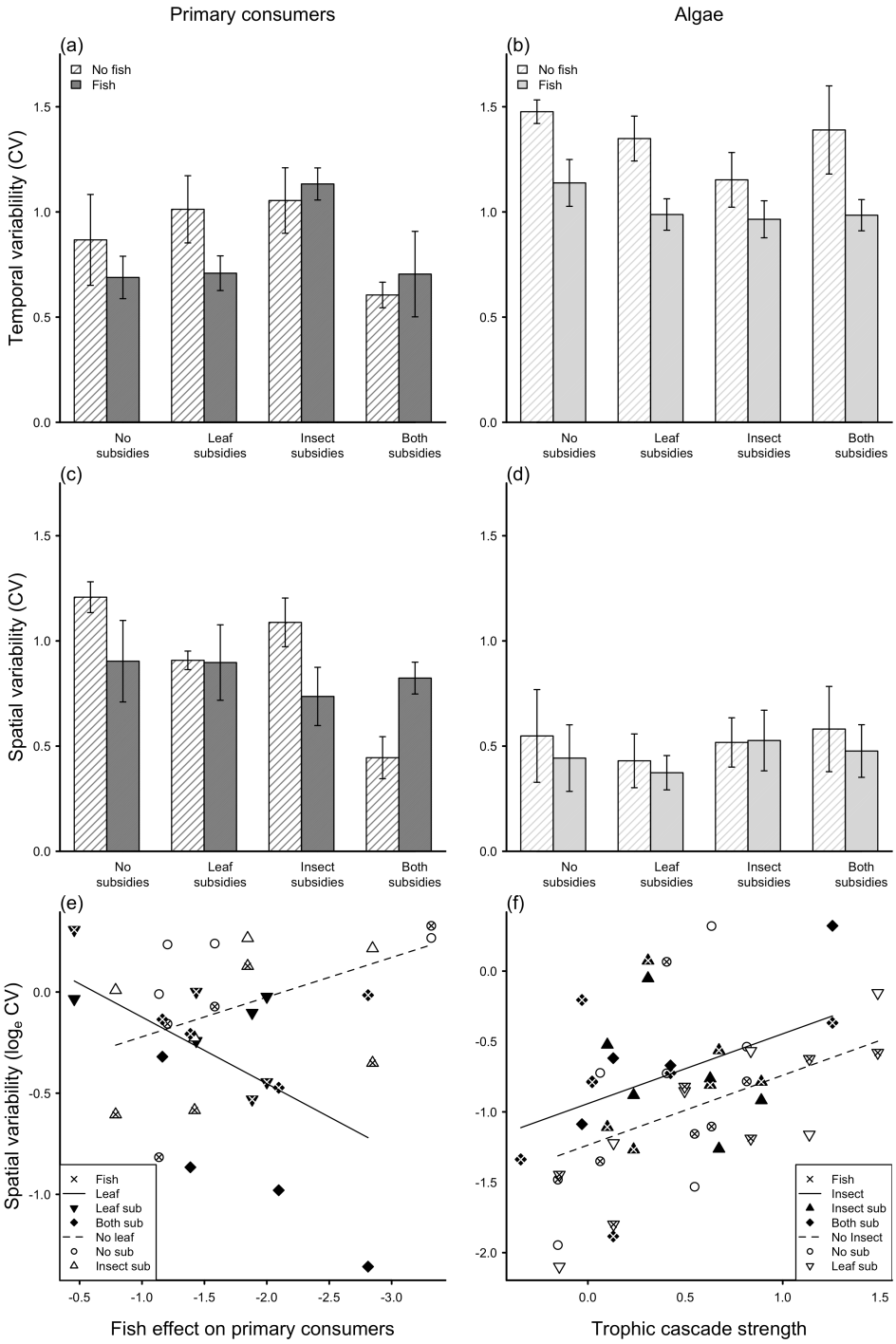


Figure 3.5: Patterns in primary consumer (a, c, & e) and algal (b, d, & f) temporal and spatial variability of biomass in response to additions of terrestrial leaf and insect subsidies. Temporal variability (+/- standard error) of fish (solid bars) and no fish (hashed bars) treatments was calculated as temporal coefficient of variation (temporal CV) for (a) primary consumer dry weight (mg/m^2) and (b) algal biomass (mg/m^2). Spatial patchiness (+/- standard error) of fish (solid bars) and no fish (hashed bars) treatments was calculated as spatial coefficient of variation (spatial CV) for (c) primary consumer dry weight (mg/m^2) and (d) algal biomass (mg/m^2). In the relationship between fish effect size (i.e. trophic cascade strength) and spatial patchiness (log$_e$-transformed CV) for (e) primary consumer dry mass (mg/m^2), solid points and solid lines are leaf subsidy treatments, open points and dashed lines are no leaf subsidy treatments, and fish treatments are marked with an “x” and (f) algal biomass (mg/m^2) where solid points and solid lines are insect subsidy treatments, open points and open lines are no insect subsidy treatments, and fish treatments are marked with an “x”.

DISUCSSION

Trophic cascade strength naturally varies and there are multiple hypotheses for causes of variation, such as consumer metabolism or primary producer generation time (Borer et al. 2005, Kurle and Cardinale 2011). In addition, resources to food-limited predators and primary consumers also have the potential to alter trophic cascades (Leibold 1989, Cebrian 1999, Polis 1999). Cross-ecosystem subsidies, because they involve a decoupling of consumer dynamics from their *in situ* resources (Polis et al. 1997), offer a powerful way to investigate top-down and bottom-up regulation within food webs. I used manipulation of cross-ecosystem subsidies to multiple trophic levels, therefore reducing food-limitation of the respective trophic levels, to investigate how reduction in food-limitation affected top-down trophic cascades and variability of primary consumer and primary producer biomass. I found that insect subsidies to top-predators increased short-term trophic cascades, whereas leaf subsidies to primary consumers increased trophic cascades through time. When both subsidies where added to food webs at the same time, trophic cascades were dampened, and primary consumer variability decreased. Interestingly, top-down effects of predators on primary producers increased primary producer spatial variability. Therefore, reduction in food-limitation of different trophic levels by subsidies altered top-down regulation on primary consumer and primary producer biomass, as well as the relationship between top-down regulation and spatial variability of biomass.

Trophic cascade strength

Cross-ecosystem subsidies can alter top-down trophic cascades through multiple mechanisms. Decreasing subsidies to top-predators can cause behavioural prey-switching to *in situ* consumers, releasing primary producers from consumption (Nakano et al. 1999). Alternatively, increasing subsidies to primary consumers, by increasing primary consumer biomass, can cause numerical increases in predator biomass that increases top-down control in the food web (Nyström et al. 2003). By altering resources to both trophic levels over 18 months, I was able to investigate how bottom-up regulation altered both short-term behaviourally-driven trophic cascades versus longer-term numerically driven trophic cascades.

Trophic cascade strength fluctuated seasonally within my tanks. With no additional resources added to food webs, trophic cascade strength varied through time and was strongest in the spring. Also, trophic cascade strength was reduced across all subsidy treatments in summer. While the direct mechanisms for these patterns are not known and were not the

focus of this study, they are potentially related to both top-down and bottom-up seasonal influences. Upland bullies spawn in the spring (McDowall and Eldon 1997), and spawning increases energy demand in fish, resulting in increased prey consumption (Wootton 1994), which may explain stronger top-down effects in my system at that time. Algal biomass was four times higher in summer than other seasons, which may have also overwhelmed the top-down effects of primary consumers at that time. Despite these strong seasonal trends, the bottom-up effects of subsidy resources to predators and primary consumers were clear (see Chapter 2) and noticeably altered trophic cascade strength.

After two months of adding resources, contrary to my prediction, the strongest trophic cascades occurred with insect subsidies to top-predators. If this was due to behavioural prey-switching by fish, then increases in primary consumer biomass would have been observed in the fish treatments. However, it was fishless tanks that had a numerical increase in primary consumer biomass, similar to my predictions for subsidies to primary consumers (Box 3.1a&c). The most plausible explanation for this pattern in fishless tanks is that insect subsidies were not consumed by fish, and therefore likely entered the detrital pathway becoming an added resource to primary consumers (Williams et al. 1993, Nowlin et al. 2007, Dreyer et al. 2012). Hoekman et al. (2011) similarly found increased detritivore biomass with increased midge carcasses entering the terrestrial detrital pathway adjacent to lakes. Likewise, additions of terrestrial arthropod carcases to pitcher plants increased detritivores in predator-free food webs (Hoekman 2007). Therefore, insect subsidies are not just subsidies to predators, but can also subsidise primary consumers, suggesting it is food-limitation of primary consumers causing these short-term trophic cascades (Box 3.1a-d).

Effects of leaf subsidies to primary consumers on trophic cascade strength increased with time, contrary to my hypothesis. I hypothesized that early in the experiment, prey-switching by primary consumers from *in situ* algal biomass to leaf subsidies, combined with lower primary consumer biomass due to fish predation, would create large increases in algal biomass in fish tanks, leading to strong trophic cascades. However, in treatments with just leaf subsidies, there was no difference between fish and fishless tanks, suggesting a lack of primary consumer prey-switching from algae to leaf subsidies. Interestingly, the strong early numerical increase in primary consumer biomass that occurred with insect subsidies was not observed with leaf subsidies over the duration of the experiment (contradicts prediction in Box 3.1a&b). Although there was not a numerical primary consumer biomass increase from leaf subsidies, there is evidence that primary consumer taxa richness increased through time in leaf subsidy treatments (Appendix 3.1), suggesting that it was a change in community

composition, and not an increase in biomass, that led to leaf subsidy top-down effects from primary consumers to algae. Also, as discussed in Chapter Two, by the end of the experiment tanks receiving leaf subsidies had a significantly different community composition compared to tanks without leaf subsidies. Moore et al. (2004) found that as leaf subsidies decompose, they generate a range of organic matter types (coarse particulate matter, fine particulate matter, dissolved organic matter) and associated resources (e.g., microbial and fungal biofilms) creating a more heterogeneous resource base, which might be a possible explanation for altered community composition, but not increased biomass, associated with leaf subsidies. Although this experiment was longer than the average experimental manipulation of subsidies (Chapter One), it was probably not long enough to have observed leaf subsidies increasing predator biomass at higher trophic levels that might have subsequently led to strong top-down effects. Therefore, it was likely the interaction between primary consumers and their food resources altered trophic cascade strength in tanks receiving leaf subsidies, opposed to the interactions between predators and primary consumers.

As predicted, when additional resources were entering the food web at the primary consumer and predator levels simultaneously, trophic cascades were absent in food webs until over 530 days had passed. This potentially could be because of a combination of the mechanisms described in the above paragraphs, where terrestrial insects and leaves were subsidising primary consumers in fish and fishless tanks. Surprisingly, there were more instances of trophic cascades throughout the year in food webs that were not receiving resources (no subsidy treatments), compared to the food webs receiving both resources (both subsidy treatment). Although the input of resources to *both* primary consumer and top-predators has been shown to decrease trophic cascades in theoretical models (Huxel et al. 2002), this is some of the first empirical evidence. My results from manipulation of insect, leaf, and both insect and leaf subsidies simultaneously suggest that it is decreased food-limitation of primary consumers, and not predators, that alters trophic cascade strength (Box 3.1a-d).

Variability of primary consumer and primary producer biomass

Under typical scenarios of consumer/resource interactions, increasing bottom-up resources can increase consumer biomass, which in turn can reduce resources, creating positive feedback loops that increase variability of consumer biomass (Abrams 1992, 2000). However, because subsidies disassemble those feed back loops, increased top-down effects

might actually decrease variability of prey guilds due to strong, food-web interactions (Box 3.1; Hillebrand 2008, O'Gorman and Emmerson 2009). Moreover, because the input of subsidies, especially in my experiment, can provide a constant resource to consumers, the bottom-up increase in resources may increase variability in biomass of consumers through runaway production (Box 3.1; Rosenzweig 1971). Therefore, I predicted if a trophic level was a) food-limited (bottom-up regulated), variability would increase or b) predator-limited (top-down regulated), variability would decrease (Box 3.1).

Temporal and spatial variability of primary consumer biomass was lowest when food webs were receiving both leaf and insect subsidies simultaneously. Furthermore, opposite to predictions (Box 3.1 c&e) spatial variability of primary consumers decreased as fish effects increased in tanks receiving leaf subsidies. Leaf and insect subsidies may have increased the detrital resource base for primary consumers, creating a detrital food web compartment. Rooney et al. (2006) found that the combination of an *in situ* food-web compartment and a subsidy driven detrital food-web compartment can stabilise consumer populations. Therefore, leaf and insect subsidies may decrease variability of primary consumer biomass by increasing the number of food-web compartments available to consumers (further discussed in Chapter 2).

Unlike effects of leaf subsidies on variability of primary consumer biomass, spatial variability in algal biomass increased with increasing top-down trophic cascades, with larger spatial variability in food webs receiving insect subsidies. This was different from my initial predictions (Box 3.1), where I proposed that increases in trophic cascades due to changes in algal biomass in fish and fishless tanks (Box 3.1m-r) would lead to variability in algal biomass not correlating to trophic cascade strength. Although fish decreased temporal variation of algal biomass, one explanation is that the release of algae from top-down forces in fish food webs through a decrease in primary consumers, allowed for runaway, bottom-up algal production leading to increased spatial variation of algal biomass. This effect could have been exacerbated if the decomposition of insect subsidies increased nutrients in the system, further increasing bottom-up effects on algal biomass. Theoretical models show that decomposers (such as bacteria) can have positive effects on primary producers by increasing nutrient availability (Daufresne and Loreau 2001), and Kratina et al. (2012) found that added nutrients and release from top-down effects during strong trophic cascades increased primary producer variation. Thus, through a release of top-down control, fish and insect subsidies increased runaway bottom-up production of algal biomass, increasing spatial variability between tanks.

Conclusions

Trophic cascades in my system appeared to be driven by food-limitation at the primary consumer trophic level. This suggests that changes in trophic cascade strength, as well as variability of primary consumer and primary producer biomass, was driven by subsidies to primary consumers, whether it be insect subsidies entering detrital food-web compartments, or leaf subsidies breaking down and increasing heterogeneity of resources. Therefore, altering subsidy trophic level, although is altering resources to predators as well as primary consumers, may only affect top-down control when altering the interactions between primary consumers and *in situ* primary producers.

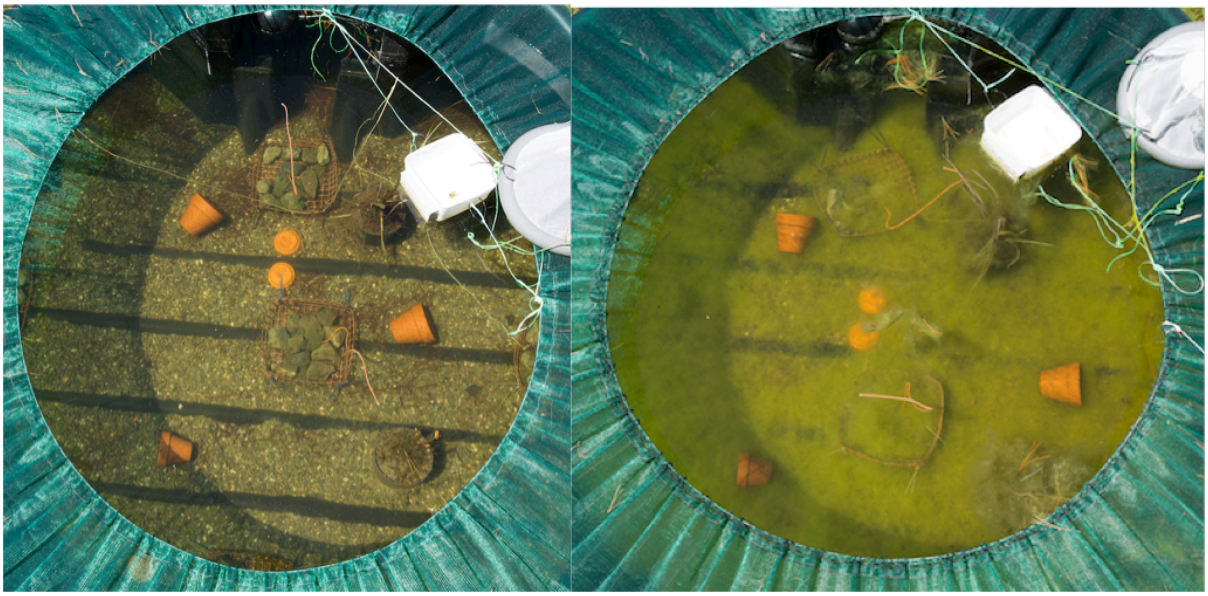


Photo: Angus McIntosh

Chapter Four: RECIPROCAL FLUXES BETWEEN TERRESTRIAL AND FRESHWATER ECOSYSTEMS ARE ALTERED BY SUBSIDIES TROPHIC LEVEL AND CONSUMER LIFE HISTORY

INTRODUCTION

Terrestrial and freshwater ecosystems are highly connected by the reciprocal exchange of subsidies (Richardson et al. 2010, Bartels et al. 2012), and consumers in both ecosystems can be reliant on resources originating in adjacent ecosystems (Baxter et al. 2005, Richardson et al. 2010, Bartels et al. 2012, Soininen et al. 2015). Subsidies from terrestrial ecosystems include many types of resources (nutrients, dissolved carbon, particulate leaf matter, arthropods, etc.; Richardson et al. 2010, Marcarelli et al. 2011, Bartels et al. 2012), but the movement of energy from freshwater to terrestrial ecosystems primarily occurs through the emergence of aquatic larvae with terrestrial adult life stages (Baxter et al. 2005, Gratton and Vander Zanden 2009, Dreyer et al. 2015). Multiple factors could potentially influence subsidies of emerging aquatic insects, including the magnitude of terrestrial subsidies to aquatic ecosystems, trophic level at which terrestrial subsidies are consumed, and aspects of aquatic food-web structure, such as the presence of top-predators that may reduce the emergence of the insects to the terrestrial ecosystem (Baxter et al. 2004, Wesner 2010, Greig et al. 2012, Kraus and Vonesh 2012). However, little is known of how these factors interact to influence subsidies from aquatic to terrestrial ecosystems.

Terrestrial subsidies to freshwater food webs could increase subsidies of emerging freshwater invertebrates through multiple mechanisms. Nutrient subsidies to freshwater ecosystems that stimulate *in situ* primary productivity can increase the biomass of freshwater invertebrates and therefore the biomass of emerging aquatic insects to terrestrial ecosystems (Greig et al. 2012). Similarly, terrestrial leaf subsidies provide resources to larval primary consumers in aquatic ecosystems, potentially increasing the biomass of emerging adult life stages from the aquatic systems (Kraus and Vonesh 2012). Finally, terrestrial arthropods are consumed by fish, which can result in prey switching that releases aquatic primary consumers from consumption, and increases their emergence, bolstering energy transfer to terrestrial consumers (Baxter et al. 2004). Although the details of these mechanisms vary, ultimately increased input of terrestrial resources to aquatic ecosystems is likely to increase the biomass of aquatic primary consumers. In aquatic ecosystems, where primary consumers are

predominately insects with complex life cycles (i.e. aquatic larval stages and terrestrial adult stages), this results in greater emergence of aquatic insects to terrestrial ecosystems (Baxter et al. 2004, Greig et al. 2012, Kraus and Vonesh 2012).

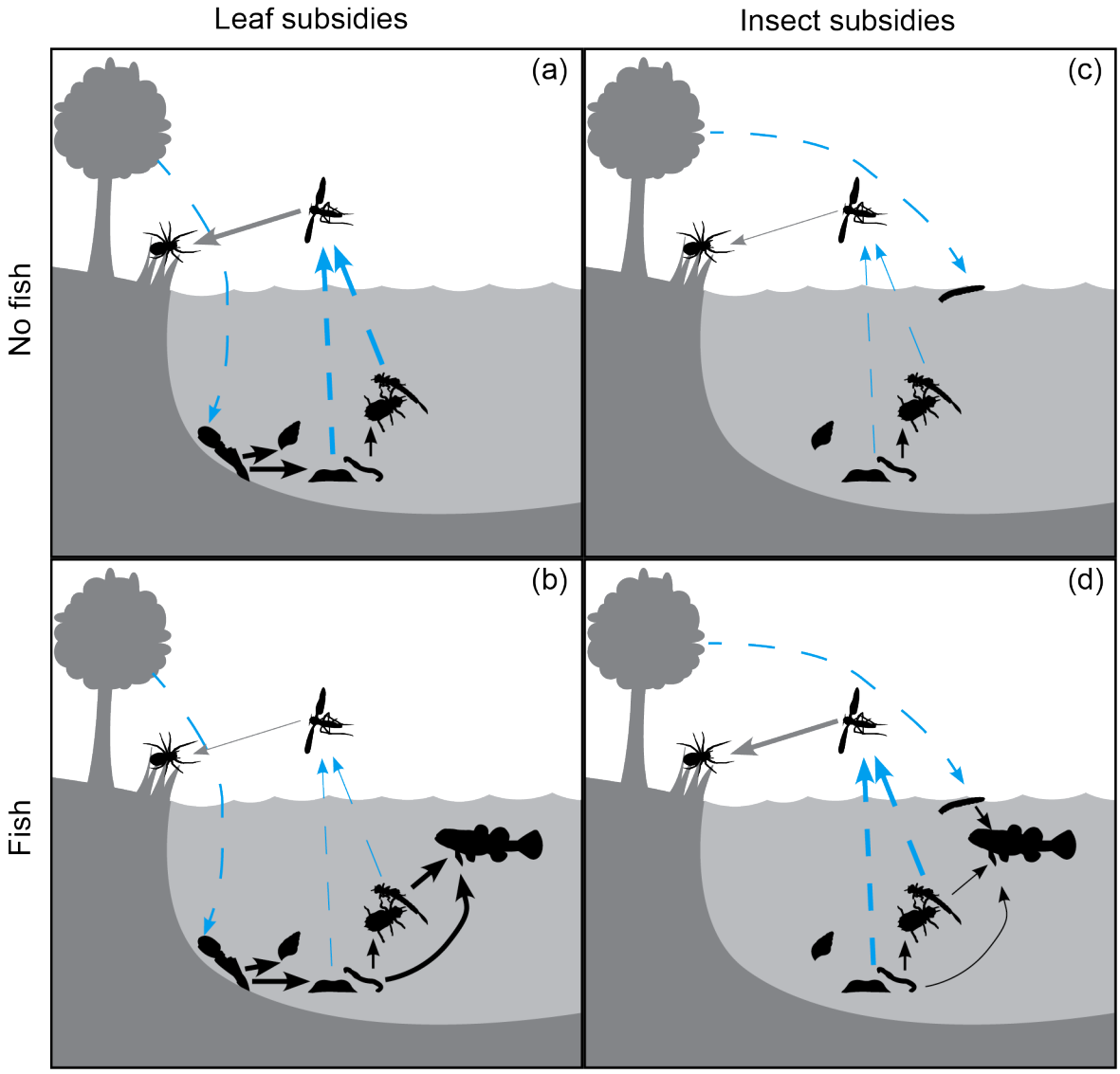


Figure 4.1: Hypotheses for how the effects of terrestrial subsidies entering at different trophic levels on aquatic insect emergence will be affected by fish presence: (a) Terrestrial leaf subsidies may increase aquatic emergence by increasing resources to primary consumers, whereas (b) the effect of leaf subsidies on emergence is likely reduced by emergence through their consumption of freshwater invertebrates, (c) terrestrial insect subsidies will be unlikely to alter emergence of freshwater organisms without fish present, (d) fish are likely to increase the effect of insect subsidies on emergence through prey-switching from feeding on *in situ* invertebrates to feeding on insect subsidies. Blue dashed arrows, subsidy flux; black arrows, energy propagation in aquatic ecosystems; and grey arrows, possible effects on terrestrial consumers not measured by this experiment. Arrow thickness, increased energy movement between organisms.

The timing of subsidies of aquatic emergence to terrestrial food webs may be just as important as the amount of the subsidy (Nakano and Murakami 2001, Uesugi and Murakami

2007). Reciprocal subsidies of insects between terrestrial and aquatic ecosystems can stabilise consumer dynamics if the subsidies are off-set in timing from *in situ* resources (Takimoto et al. 2002), partially because they provide critical resources at times of *in situ* resource scarcity. Nakano and Murakami (2001) found that inputs of insect subsidies from terrestrial to freshwater systems occurred primarily when the abundance of *in situ* freshwater primary consumers was low. Similarly the emergence of freshwater insects to terrestrial ecosystems occurred during a period of low abundance of *in situ* terrestrial arthropods prior to leaf set in deciduous forests. Therefore, the timing of subsidies between terrestrial and aquatic ecosystems could potentially stabilise interactions within both ecosystems (Nakano and Murakami 2001).

Despite their potential importance, the transfer of energy subsidies from freshwater to terrestrial ecosystems may not be realised if cross-ecosystem bottlenecks in freshwater food webs prevent emergence of aquatic insects as adults. Freshwater ecosystems could become sinks of terrestrial energy if organisms that do not have a terrestrial adult stage, such as snails and fish, consume the extra resources. These carbon sinks can be thought of as cross-ecosystem bottlenecks, whereby the flow of energy among adjacent ecosystems is interrupted by carbon storage in immobile organisms. Primary consumers that have fully aquatic life cycles, such as snails, can increase with the addition of terrestrial leaf subsidies (Klemmer and Richardson 2013), preventing the return of those resources, or the transfer of freshwater primary production, to the terrestrial ecosystem. Predatory fish reducing emergence, by either consumption of aquatic larvae or their emerging adults, are another potential bottleneck (Wesner 2010, Greig et al. 2012). Therefore, life histories of aquatic consumers within recipient ecosystems may determine whether the exchange of energy between aquatic and terrestrial ecosystems is reciprocal. Furthermore, the balance between increased resource availability and cross-ecosystem bottlenecks could determine the extent to which freshwater habitats are resource sources or sinks in linked aquatic-terrestrial systems.

To investigate this interaction between different terrestrial subsidies on aquatic insect emergence, I ran an experiment crossing the presence of terrestrial leaf subsides to primary consumers, the presence of terrestrial insect subsidies to fish, and the presence of fish in freshwater pond mesocosms over 18 months. I hypothesized that leaf subsides would increase aquatic primary consumer biomass, increasing subsequent subsidies to terrestrial ecosystems through aquatic insect emergence, resulting in reciprocal exchanges (Figure 4.1a). However, if the increase in primary consumer biomass was primarily in the form of organisms without complex-life cycles like snails, possibly because they are more tolerant or competitive under

high resource levels, then emergence would decrease, potentially creating cross-ecosystem bottlenecks. I also predicted that presence of predatory fish would decrease emergence through consumption of primary consumers, and therefore increase the likelihood that aquatic ecosystems act as sinks of terrestrial energy (Figure 4.1b). In contrast, I hypothesized that subsidies of terrestrial insects would cause fish to prey-switch, reducing their consumption of aquatic primary consumers, and increasing primary consumer emergence (Figure 4.1d). However, I didn't expect this to happen in fishless ponds because the fish that consume subsidies of terrestrial insects are absent (Figure 4.1c). Finally, I anticipated an additive increase in aquatic emergence when both terrestrial leaf and insect subsidies were entering the freshwater food web simultaneously, due to decreased fish predation on aquatic primary consumers combined with increased resources to those same primary consumers.

METHODS

Experimental set-up

The 18-month pond mesocosm experiment took place in the Southern Alps, South Island, New Zealand at the University of Canterbury's Cass Mountain Research Station. In January 2013, thirty-two 1,100 litre cattle tanks (mesocosms) were filled with ground water, 1 cm of gravel substrate, common pond macrophytes from the area (submergent *Myriophyllum* and emergent *Carex*), and two 10-cm diameter ceramic pots positioned on their side to provide fish habitat. Experimental tanks were inoculated with 5 l of filtered pond water, 380 ml aliquots of concentrated phytoplankton and zooplankton, and 300 ml filtered fine particulate organic matter. To ensure representation of various trophic levels and feeding groups, a range of benthic invertebrates from local ponds and lakes were added to tanks in natural densities based on exploratory surveys (predatory invertebrates: 10 *Procordulia* dragonflies and 10 *Xanthocnemis* damselfies; primary consumers: ~200 *Potamopygrus* snails, ~100 Chironominae midges and 50 *Triplectides* caddisflies). In addition to those taxa, two 0.3 m^2 sweeps of benthos with a 1-mm mesh D-net from local ponds (one from a permanent pond and one from a temporary pond) were added to the tanks to increase diversity of rare taxa to mimic naturally occurring food webs. Tanks were left to be naturally colonised by terrestrial dispersal of adult stages of invertebrates from January to May 2013 before experimental treatments were added; natural colonization continued to occur throughout experiment.

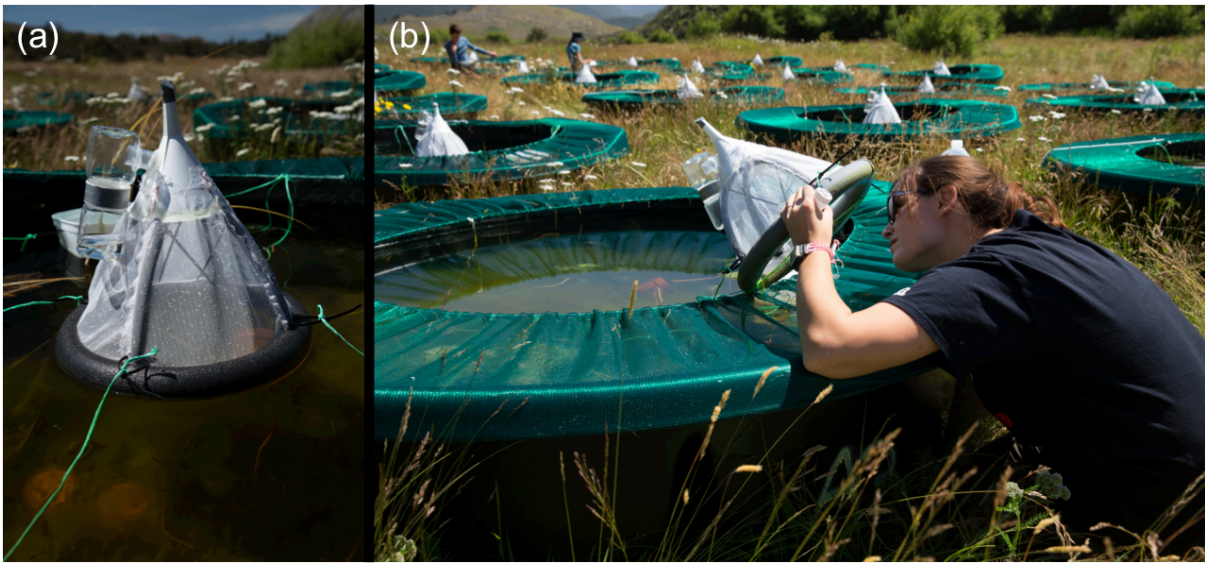


Figure 4.2: Emergence traps (a) on experimental tanks and (b) methods for collecting emerging invertebrates that were too big for the collection jar. Photo: Angus McIntosh.

The experiment had a fully crossed 2 x 2 x 2 factorial design, with the presence/absence of subsidies to primary consumers (leaves) and the presence/absence of subsidies to top-predators (beetle larvae) crossed with the presence/absence of top-predators (fish). Each treatment was replicated four times and randomised within four spatial blocks of tanks. Ten adult upland bullies (*Gobiomorphus breviceps*) (totalling 26.6 +/- 0.4 g), a common native predatory fish in New Zealand freshwater ecosystems (Staples 1975), were added as the top predator to "fish" treatment tanks on 14 May 2013. These adult fish reproduced in December 2013, and young-of-the-year (YOY) upland bullies were present from this point onward.

Four grams of air-dried riparian willow leaves (*Salix fragilis,* the most common riparian tree in the area) were added as resources to primary consumers ("leaf subsidy") every four weeks, beginning 22 May 2013. Every two weeks, beginning 22 May 2013, two grams of live beetle larvae (*Tenebrio* sp.) were added as resources to predatory fish ("insect subsidy"). Beetle larvae were used so I could easily control subsidy additions.

Emergence sampling

To measure the effects of terrestrial subsidies on aquatic emergence, I sampled adult emerging aquatic invertebrates from the tanks during the spring and summer of 2013-14. Emergence traps were first deployed in late spring (15 November 2013), 178 days after the experiment began, during the period where emergence was expected to begin increasing towards the summer peak. Adult invertebrates were collected from traps every two to four

weeks, for a total of seven sampling dates (days 203, 232, 247, 264, 274, 298, and 322 of the experiment). Floating emergence traps, loosely tethered over the center of the tanks (covering 0.08 m^2 surface area), were constructed with 1-mm mesh and a collection jar containing 50% ethanol to preserve invertebrates (Figure 4.2 a). Large emerging invertebrates, such as Odonata, were sometimes found attached to mesh on the inside of the trap rather than in the collection jar, so traps were carefully inspected for all emerged invertebrates during each sampling occasion (Figure 4.2 b). Invertebrates were preserved in 70% ethanol until later processing in the laboratory.

In the laboratory, adult aquatic invertebrates collected from the traps were identified to family or order, then photographed using a Leica DFC450 microscope camera. Length measurements were taken by uploading the digital photographs to Adobe Acrobat Pro where the 'Measuring Distance' tool was used. Lengths were converted to dry mass using length-weight regressions by Sabo et al. (2002) and Stagliano et al. (1998). The adult invertebrates were categorized as predatory invertebrates or primary consumers based on their aquatic larval stages (following Greig 2008).

The ratio of non-emergent to emergent primary consumer biomass for tanks was calculated using final biomass estimates of primary consumer taxa from Chapter 2; taxa included in the non-emergent category were: Oligochaeta, *Gyraulus* sp., Planorbidae, *Physa* sp., *Potamopyrgus* sp., and Sphaeriidae. All other primary consumers had complex life histories and were included in the emergent category.

STATISTICAL ANALYSIS

Total emerged biomass was calculated by summing all emergence from a tank over the duration that emergence traps were deployed. Biomass was calculated for all invertebrates, as well as predatory invertebrates and primary consumers separately. Mixed-effects models on $\log_e$-transformed biomass (total, predatory invertebrate, and primary consumer), as well as non-emergent to emergent ratio of primary consumer biomass, were conducted with experimental block as a random factor and terrestrial leaf subsidy presence, terrestrial insect subsidy presence, fish presence, and their interactions as fixed factors.

Emergence rate ($mg/m^2/day$) was calculated over the course of the collection period by dividing total biomass from each collection by the number of days since the last collection. Time-series analysis on emergence rate was conducted using mixed-effects models with tank nested within experimental block as random factors and terrestrial leaf subsidy presence, terrestrial insect subsidy presence, fish presence, sampling date, and their

interactions as fixed factors. Including moving average autocorrelation into the mixed effects model (ARC1 function; Bence 1995) did not improve model fit, so was not included in the final model. *Post-hoc* mixed-effects models on emergent biomass rate for each sampling date were run with terrestrial leaf subsidy presence, terrestrial insect subsidy presence, fish presence, and their interactions as fixed effects with experimental block as a random effect. All mixed effects models were conducted using 'nlme' package (Pinheiro et al. 2015) in R (R Core Team 2014).

Table 4.1: Results of mixed-effects linear models for (a) total emerged biomass (mg/m^2), (b) emerged predatory invertebrate biomass (mg/m^2), and (c) emerged primary consumer biomass (mg/m^2) with presence of insect subsidies, presence of leaf subsidies, presence of fish, and their interactions as fixed factors and experimental block as a random factor. Degrees of freedom = 1,21 for all predictor terms. Bold p-values are significant (alpha = 0.05).

Variable	F	P	analysis
a) Total emereged biomass (mg/m^2)*			Mixed effects
insect subsidies	9.58	**0.005**	
leaf subsidies	1.57	0.223	
fish	20.76	**<0.001**	
insect x leaf	4.47	**0.046**	
insect x fish	1.09	0.307	
leaf x fish	3.26	0.085	
insect x leaf x fish	0.01	0.943	
b) Predatory invertebrates (mg/m^2)*			Mixed effects
insect subsidies	4.67	**0.042**	
leaf subsidies	0.01	0.973	
fish	42.39	**<0.001**	
insect x leaf	2.84	0.106	
insect x fish	0.03	0.866	
leaf x fish	0.51	0.483	
insect x leaf x fish	0.06	0.815	
c) Primary consumers (mg/m^2)*			Mixed effects
insect subsidies	9.17	**0.006**	
leaf subsidies	4.00	0.058	
fish	0.33	0.566	
insect x leaf	1.74	0.201	
insect x fish	1.66	0.211	
leaf x fish	2.59	0.122	
insect x leaf x fish	0.16	0.686	

* $\log_e$-transformed data

RESULTS

Subsidies of terrestrial insects significantly increased the total biomass of emerging aquatic insects (insect subsidy main effect, p=0.005; Table 4.1a), but this effect was weakened in the presence of terrestrial leaf subsidies (insect x leaf subsidy interaction, p=0.046; Table 4.1a; Figure 4.3a). Fish significantly decreased emergence of aquatic insects

independent of any other influences (fish main effect, p<0.001 and no significant interaction involving fish; Table 4.1a; Figure 4.3a). These patterns were partially mirrored in total predatory invertebrate emergence. Emergence of predatory invertebrates significantly increased with insect subsidies (insect subsidy main effect, p=0.042; Table 4.1b), with almost four times the biomass emerging compared to tanks not receiving insect subsidies (Figure 4.3b). However, in this case there was no interaction between the input of leaf and insect subsidies (insect x leaf interaction, p=0.106; Table 4.1b). Fish significantly decreased emergent biomass of predatory invertebrates (fish main effect, p<0.001; Table 4.1b), with tanks without fish having six times the biomass of predatory invertebrate emergence compared to those with fish (Figure 4.3b). Interestingly, there was no significant effect of fish presence on primary consumer emergence (fish main effect, p=0.566; Table 4.1c; Figure 4.3c) even though the proportion of primary consumers that could emerge (i.e. insects with complex, terrestrial/aquatic life cycles) was significantly greater in fish tanks (fish main effect, p=0.022; Appendix 4.1). Thus, the fish effect on total emergence was almost totally associated with their effect on predatory insects. Finally, similar to the predatory invertebrates, there was significantly higher primary consumer emergence in tanks receiving insect subsidies (insect subsidy main effect, p=0.006; Table 4.1c; Figure 4.3c).

Table 4.2: Results of mixed-effects linear models for the rate of emergence, measured as biomass per-day, with presence of insect subsidies, presence of leaf subsidies, presence of fish, date, and their interactions as fixed factors and tank nested within experimental block as a random factors. F-values are presented as F-value with predictor and error degrees of freedom as subscript for all predictor terms. Bold p-values are significant (alpha = 0.05).

Variable	F	P	analysis
Total emereged biomass (mg/trap)*			Mixed effects
insect subsidies	$3.97_{(1,21)}$	0.059	
leaf subsidies	$0.68_{(1,21)}$	0.418	
fish	$6.89_{(1,21)}$	**0.015**	
date	$13.43_{(6,25)}$	**<0.001**	
insect x leaf	$9.08_{(1,21)}$	**0.006**	
insect x fish	$0.01_{(1,21)}$	0.942	
leaf x fish	$1.37_{(1,21)}$	0.254	
insect x date	$1.95_{(6,125)}$	0.077	
leaf x date	$0.59_{(6,125)}$	0.732	
fish x date	$5.00_{(6,125)}$	**<0.001**	
insect x leaf x fish	$1.76_{(1,21)}$	0.198	
insect x leaf x date	$1.06_{(6,125)}$	0.389	
insect x fish x date	$1.55_{(6,125)}$	0.167	
leaf x fish x date	$0.66_{(6,125)}$	0.685	
insect x leaf x fish x date	$1.27_{(6,125)}$	0.272	

* $\log_e$-transformed data

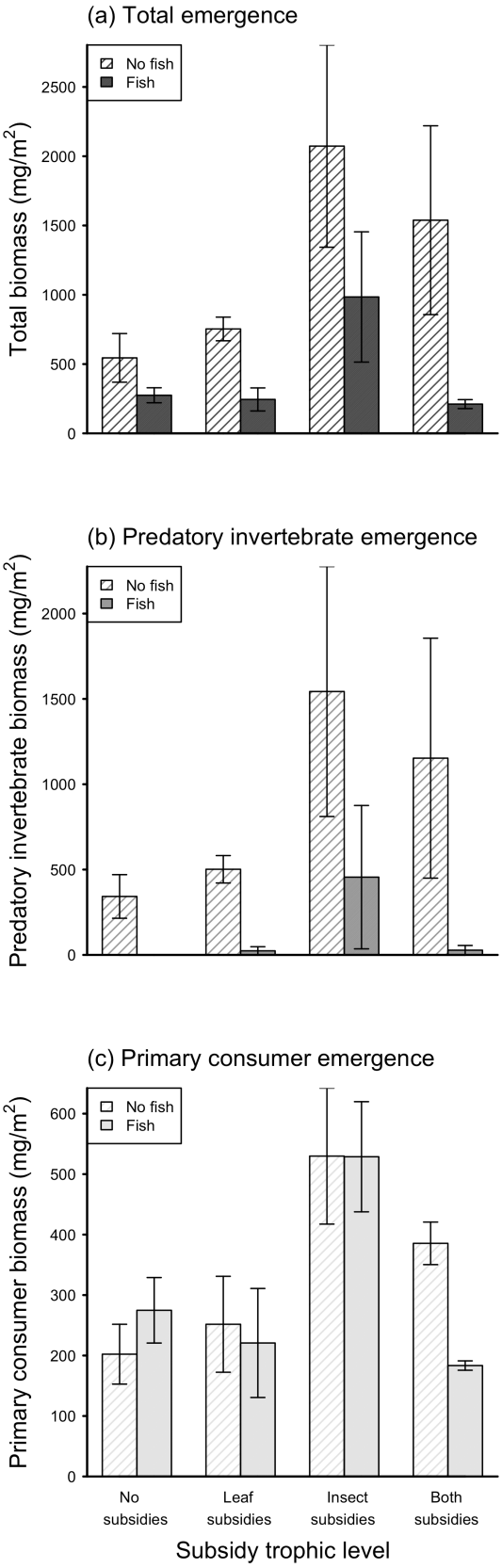


Figure 4.3: Mean biomass (mg/m^2) of emerging (a) invertebrate total, (b) predatory invertebrates, and (c) primary consumers for fish and fishless treatments receiving either no subsidies, terrestrial leaf subsidies, terrestrial insect subsidies, or both insect and leaf subsidies. Error bars are +/- 1 standard error of the mean.

Fish significantly affected the daily rate of emergence from the tanks, with the direction of this fish effect varying significantly through time (fish x time interaction, $p<0.001$; Table 4.2; Figure 4.4). Post-hoc mixed-effects models on individual dates revealed that fish significantly deceased aquatic insect emergence rate from days 203 through 247 of the experiment (fish main effect day 203, $p=0.001$; day 232, $p=0.005$; day 247, $p=0.017$; Appendix 4.3a-c; Figure 4.4). However, the negative effect of fish on insect emergence diminished after day 264 (Appendix 4.3d-g; Figure 4.4), and on day 274 fish actually increased emerging biomass in tanks receiving insect subsidies (significant fish by insect subsidy interaction day 274, $p=0.003$; Appendix 4.3e; Figure 4.4c&d). Thus, although fish reduced the total biomass of emerged insects, the nature of their influence varied over time.

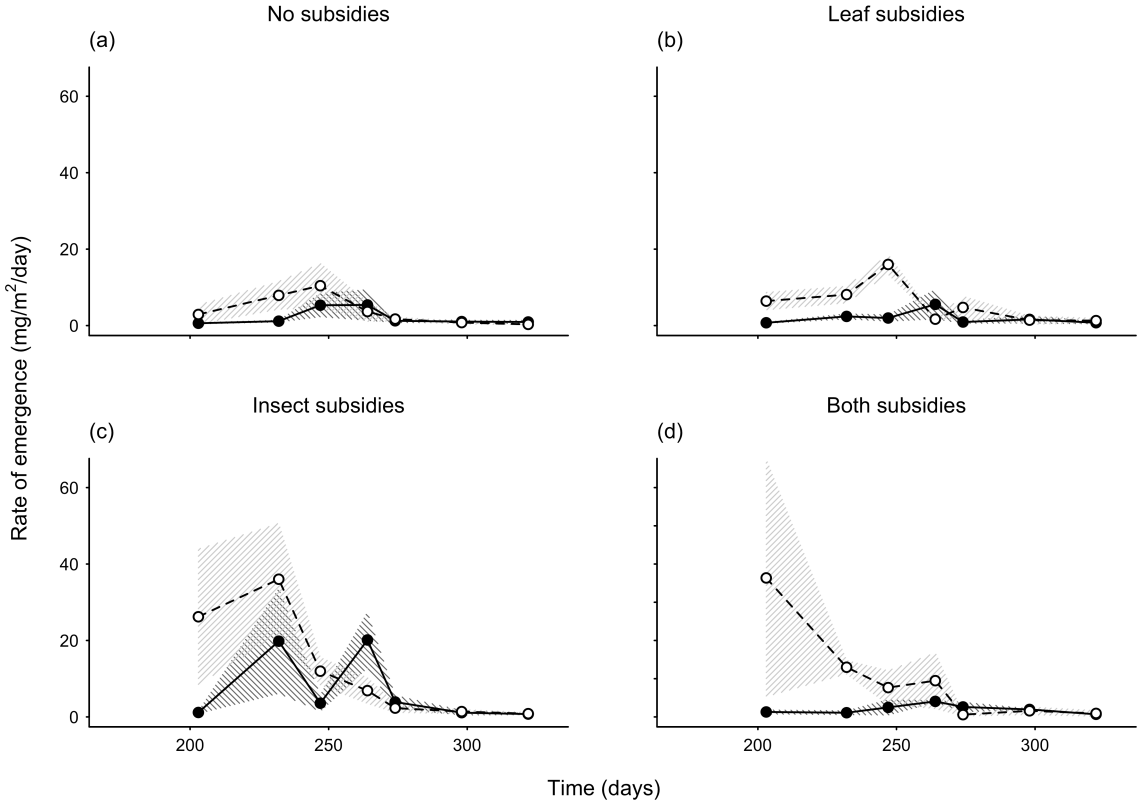


Figure 4.4: Rate of emergence of freshwater invertebrates per-day (mg/m^2/day) over time from tanks receiving (a) no subsidies, (b) terrestrial leaf subsidies, (c) terrestrial insect subsidies, or (d) both leaf and insect subsidies. Open points with dashed lines are treatments without fish and associated light grey polygons represent +/- 1 standard error of the mean (S.E.). Filled points and solid lines are treatments with fish and associated dark grey polygons represent +/- 1 S.E.

Subsidies of terrestrial insects and leaves also altered the daily rate of aquatic emergence, independent of fish. The positive effects of insect subsidies on the rate of aquatic insect emergence were significantly reduced by the presence of leaf subsidies (insect x leaf subsidy interaction, $p=0.006$; Table 4.2; Figure 4.4c&d). Insect subsides increased emergence

rate from the tanks after 203 and 232 days of the experiment (significant insect subsidy main effects day 203, p=0.021; day 232, p=0.029; Appendix 4.3a&b; Figure 4.4c&d), with leaf subsidies reducing the positive effect of insect subsidies on emergence rate on days 232 and 264 (insect by leaf subsidy interaction day 232, p=0.016; day 274, p=0.011; Appendix 4.3b&e).

DISCUSSION

Adjacent ecosystems are very often highly connected by the reciprocal exchange of energy subsidies (Polis et al. 1997, Baxter et al. 2005, Richardson et al. 2010, Bartels et al. 2012). The input of subsides from terrestrial ecosystems to multiple trophic levels within freshwater food webs has the potential to increase reciprocal transfer of subsidies back to terrestrial food webs through the emergence of freshwater invertebrates (Baxter et al. 2004, Greig et al. 2012, Kraus and Vonesh 2012). However, I hypothesised that bottlenecks in this cross-ecosystem transfer could occur if terrestrial subsidies contributed to biomass of freshwater organisms with strictly aquatic life-histories, such as snails and fish (Wesner 2010, Klemmer and Richardson 2013), thereby diminishing reciprocal subsidy exchange and creating resource sinks within freshwater ecosystems (Gravel et al. 2010). I found tanks receiving terrestrial insect subsidies were sources of aquatic insect emergence and created a reciprocal exchange of energy between aquatic and terrestrial ecosystems. In contrast, terrestrial leaf subsidies did not increase aquatic emergence, creating a sink of terrestrial resources. Furthermore, aquatic emergence was reduced by resources being usurped by organisms with completely aquatic life histories, such as fish and snails, creating cross-ecosystem bottlenecks. The mechanisms underlying these effects will be discussed below. My results suggest that, although increasing high-energy subsidies (such as insects) to a recipient ecosystem can increase reciprocal subsidies, the consumption of those subsidies by organisms that do not cross ecosystem boundaries may create cross-ecosystem bottlenecks that prevent the reciprocal exchange of subsidies. These bottlenecks to reciprocal subsidies have the potential to generate cross-system cascades by altering interactions within both ecosystems.

Increases in aquatic emergence

My experiment revealed that not all terrestrial subsidies increase aquatic insect emergence. The input of terrestrial insect subsidies increased freshwater insect emergence thereby creating reciprocal energy transfer to terrestrial ecosystems, whereas leaf subsidies dampened these effects. Although past studies also found that terrestrial insect subsidies

increased freshwater emergence, the mechanisms of those outcomes were linked to fish prey-switching, whereby fish fed on the terrestrial insects, releasing aquatic insects from consumption (Baxter et al. 2004). Those mechanisms might be applicable to my treatments with fish, but in treatments without fish, increased aquatic emergence was more likely a result of terrestrial insects entering detrital pathways and becoming high-energy food resources for primary consumers. This was observed by Nowlin et al (2007), in a study of periodical cicada emergence as subsidies to ponds, where inputs of cicadas not consumed by fish entered detrital pathways and increased primary consumer abundance. Therefore, increases in aquatic insect emergence in treatments with terrestrial insect subsidies and without fish, may be due to insect subsidies providing additional detrital resources to aquatic primary consumers.

Interestingly, leaf subsidies by themselves did not increase emergence, and reduced the positive effects of insect subsidies on emergence. This is likely due to changes in invertebrate community composition brought about by increased leaf detritus (discussed further in Chapter Two). Some of the main components of altered community composition with leaf subsidies were increases in *Physa* sp. and *Gyraulus* sp. snails (Chapter Two), which of course do not have terrestrial life histories. Trophic transfer to species that do not emerge from freshwater ecosystems likely creates a bottleneck, restricting reciprocal energy transfer with terrestrial ecosystems. Nonetheless, because some leaves and insect detritus would have been ultimately consumed by the same trophic level (i.e. primary consumers) within fishless treatments, effects on subsequent aquatic emergence were more likely a result of the quality of the subsidy (high-energy insect detritus subsidies versus low-energy leaf detritus), rather than the trophic level at which the subsidies were entering. Thus, resource quality is likely to be an important aspect of cross-ecosystem subsidy transfer, influencing both recipient food webs and the exchange of energy (Marcarelli et al. 2011).

Timing of emergence

My results revealed that subsidies of terrestrial insects created a pronounced temporal peak in emergence of aquatic insects in late spring/early summer, whereas all other treatments showed more consistent emergence over time. While I did not measure the impact of this emergence peak on terrestrial consumers, other studies found that timing of emergence could potentially be crucial to terrestrial consumers (Nakano and Murakami 2001, Uesugi and Murakami 2007). For example, Uesugi and Murakami (2007) found that the distribution of birds, in riparian and upland forests, shifted from upland to riparian areas during periods of

high aquatic insect emergence to consume emerged aquatic insects. Therefore, in my study, the peak in aquatic emergence from subsidies of terrestrial insects could have far reaching consequences for terrestrial consumers, potentially causing terrestrial consumers to shift distributions to take advantage of increased resources.

Cross-ecosystem bottlenecks

Although I found that terrestrial insect subsidies increased the flux of aquatic insects back to terrestrial ecosystems, fish presence reduced aquatic emergence, creating sinks of terrestrial resources in aquatic habitats. Fish can reduce aquatic emergence to terrestrial ecosystems either by consuming the *in situ* insects that would have eventually emerged (Baxter et al. 2004) or by preventing the aquatic insects with winged adults from colonizing the freshwater ecosystems in the first place (Vonesh et al. 2009). Either way, fish may act as gatekeepers by preventing additional resources from terrestrial ecosystems from being recycled via freshwater emergence back to the terrestrial ecosystem.

I observed particularly strong effects of fish on the emergence of large-bodied predatory invertebrates, which is consistent with the generally observed positive size-selective predation by fish on aquatic taxa, and their disproportionate impacts on populations of large-bodied aquatic insects (Brooks and Dodson 1965, Merrick et al. 1992, Knapp et al. 2001). Interestingly, I found that fish did not reduce emergence of primary consumers from freshwater ecosystems. This could be because fish presence altered the ratio of non-emergent to emergent primary consumer biomass. Not all primary consumers within lentic habitats, such as snails and worms that spend their whole life in the freshwater ecosystem, have complex life histories. I found that tanks without fish had a greater proportion of primary consumers that do not have an adult terrestrial stage than tanks with fish, primarily driven by a much higher abundance of snails in fishless tanks (Appendix 4.1). Thus, it is likely that the prevalence of primary consumers without terrestrial adult stages in fishless treatments created a cross-ecosystem bottleneck to the extent matched by fish predation on emerging insects in fish treatments, resulting in similar emergence of aquatic insects in fish and fishless tanks. Emergence of aquatic insects could, therefore, be reduced by bottlenecks at primary consumer and top-predator levels in aquatic ecosystems.

Conclusions

Although reciprocal subsidies play a crucial role in the stability of both terrestrial and freshwater ecosystems (Nakano and Murakami 2001, Takimoto et al. 2002), my results

indicate their relative fluxes are not independent, because terrestrial subsidies to the aquatic environment can either increase or decrease reciprocal exchanges, depending on the aquatic trophic level which consumers of the terrestrial subsidies. Moreover the presence of consumers with purely aquatic life histories can create bottlenecks that prevent the reciprocal exchange of subsidies between the two systems. Ultimately, whether freshwater ecosystems will be sources or sinks of terrestrial subsidies depends on both the relative composition of consumers with and without terrestrial adult stages and quality and trophic level of terrestrial subsidies. Thus, changes in the relative composition of both donor and recipient communities can alter both the fluxes and stocks of energy in highly connected ecosystems.

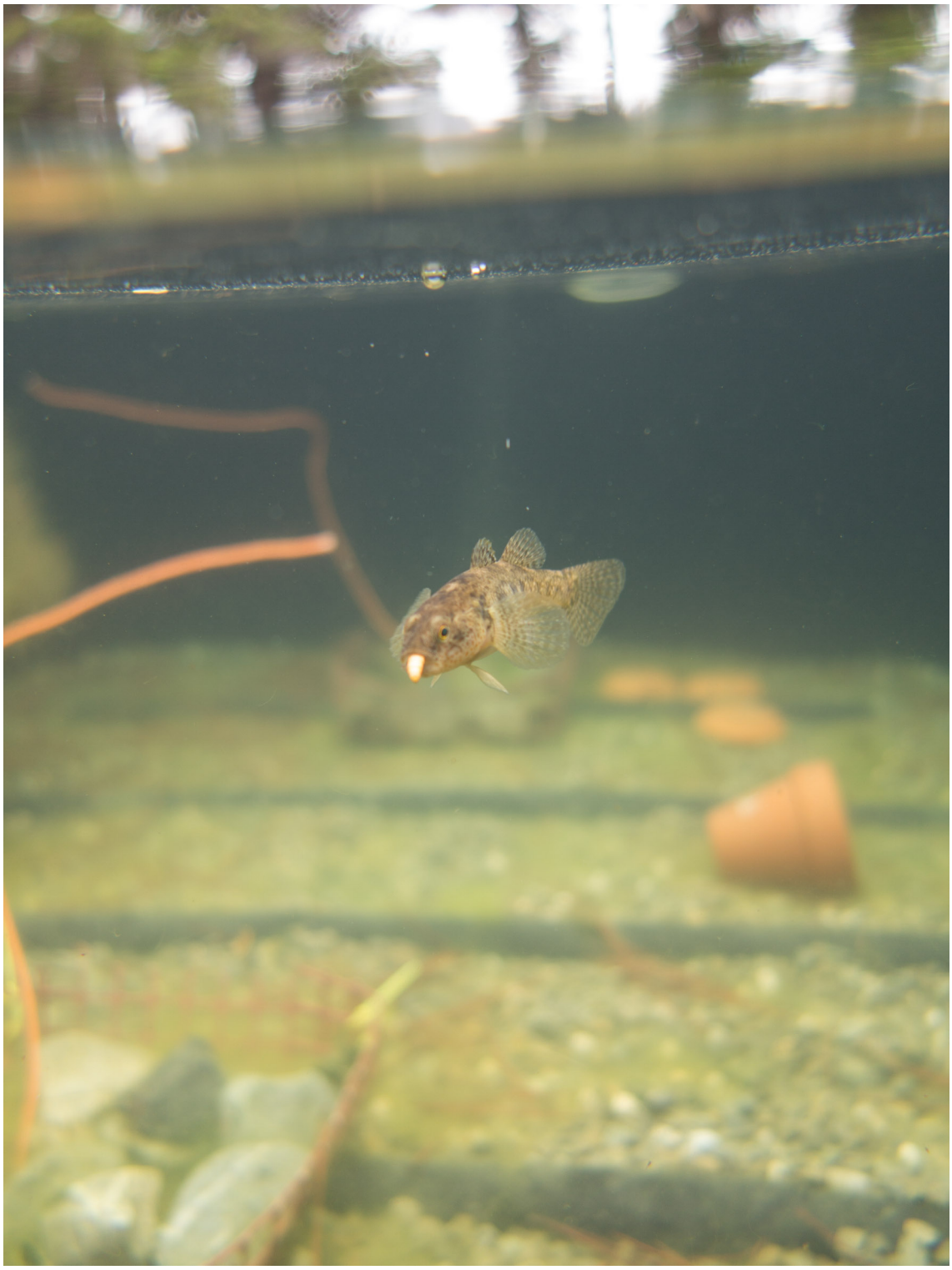


Photo: Angus McIntosh

Chapter Five: INSIGHTS GAINED FROM MANIPULATING SUBSIDY TROPHIC LEVEL

INTRODUCTION

Cross-ecosystem subsidies have the potential to not only directly influence consumers in the recipient ecosystem, but cascade through food webs to alter bottom-up and top-down interactions, and subsidy exchange between connected ecosystems (Polis et al. 1997, Baxter et al. 2005, Richardson et al. 2010). However, evidence from empirical research, meta-analyses, and theoretical models varies on exactly how subsidies alter food-web interactions (Huxel and McCann 1998, Marczak et al. 2007, Leroux and Loreau 2008, Marcarelli et al. 2011, Bartels et al. 2012, Hagen et al. 2012a). Ecosystem type (Marczak et al. 2007, Bartels et al. 2012), recipient ecosystem productivity (Marczak et al. 2007), and subsidy quantity and quality (Marcarelli et al. 2011) are all factors proposed to alter the magnitude of subsidy effects on recipient ecosystems. In addition to these mechanisms, the trophic level at which a subsidy enters the recipient ecosystem has the potential to alter food webs, from top-predators down to primary producers. Although subsidy input to various trophic levels has been studied in isolation (Nakano et al. 1999, Henschel et al. 2001, Piovia-Scott et al. 2011, Klemmer and Richardson 2013), the effects of subsidies entering multiple trophic levels simultaneously has received little attention (but see models of Huxel et al. 2002).

Through meta-analysis and experimental manipulation, I investigated how subsidy trophic level altered bottom-up and top-down interactions within recipient food webs, as well as the reciprocal exchange of subsidies back to donor ecosystems. Meta-analysis results from Chapter 1 revealed multiple knowledge gaps in empirical research on how subsidy trophic levels affect food-web interactions. These included: 1) interactive effects of subsidies entering at two separate trophic levels; 2) short-term versus long-term effects of subsidies on bottom-up and top-down interactions; and 3) how subsidy input to a system affects subsidy output. Below I will describe how the results from Chapters 2-4 increase the understanding in those three areas while highlighting remaining knowledge gaps. I will also discuss the implications of my results for anticipating the response of meta-ecosystems to increasing levels of anthropogenic change.

INTERACTIVE EFFECTS OF MULTIPLE SUBSIDIES

My meta-analysis results revealed that, to date, there has been a lack of research on the interactive effects of subsidies entering recipient food webs at two trophic levels (as discussed in Richardson and Sato 2015). Results from my experiment revealed largely additive effects of subsidies to predators and subsidies to primary consumers on biomass within food-web trophic levels; significant interactive effects of leaf and insect subsidies occurred in only one response variable. Thus, the overwhelming evidence pointed towards different subsidies having independent influences on recipient food webs within aquatic systems. Interestingly, this was not the case with the biomass of aquatic insects emerging from the tanks. In this case, leaf subsidies dampened the positive effect of insect subsidies on both total emergent biomass and rate of emergence. This is surprising given that there was not a significant dampening effect of leaf subsidies on invertebrate standing stock biomass within the tanks around the time of measured emergence. These results may indicate that the interactive effects of multiple subsidies on standing stock biomass were not captured because the biomass was being exported to the terrestrial ecosystem via insect emergence. Quantifying the gross biomass effects from added resources may be particularly difficult in aquatic systems because, depending on the time of year, the majority of aquatic insect biomass may either be present in the aquatic system or the biomass may be split between non-emergent taxa in the aquatic system and taxa that have emerged to the terrestrial ecosystem. Future studies investigating food-web interactions on freshwater invertebrates should consider, not only measuring standing stock biomass of non-emerged invertebrates, but also including emergence measurements to better understand freshwater food-web dynamics.

Models predict that subsidies consumed by predators and primary consumers together can dampen trophic cascades and increase stability within food webs (Huxel et al. 2002). However, until now, that prediction has remained untested within empirical food webs. In my experiment, I found that the top-down effect of predators on primary producers (i.e. trophic cascades) was damped by the simultaneous input of subsidies to predators and primary consumers, when compared to trophic cascade strengths in tanks that were receiving just insect or just leaf subsidies. I also found evidence of combined subsidy input decreasing temporal variability of primary consumers. These effects could be due to constant resource availability throughout the year to the top and bottom of the food web reducing food-limitation of consumers, therefore decreasing their top-down effects. This finding concurs with work by Huxel et al. (2002), who proposed that subsidies to predators would reduce top-

down effects on primary consumers, while subsidies to primary consumers at the same time would increase primary consumer biomass, therefore stabilizing fluctuations in consumer biomass through time. These results have important implications for future research on the effects of subsidies on food-webs, because if a food web naturally receives subsidies to multiple trophic levels, which is a common phenomenon (Zhang and Richardson 2011, Bartels et al. 2012, Hagen et al. 2012a), then studying the effect of one subsidy in isolation will provide an unrealistic picture of food-web dynamics.

EFFECTS OF STUDY DURATION ON BOTTOM-UP AND TOP-DOWN INTERACTIONS

Models also predict that increasing subsidies to any trophic level will lead to bottom-up propagation of those resources up the food web, increasing predator biomass. This increase in predator biomass then leads to strong top-down interactions and increasing trophic cascade strength (Leroux and Loreau 2008). I found that after 18 months of subsidy manipulation, there were varying effects of subsidies on consumer biomass. Leaf and insect subsidies propagated up foodwebs and increased biomass of intermediate predators. However, top predator (adult fish) biomass only increased with insect subsidies. Although my experiment duration was longer than the average experimental manipulation from the meta-analysis (41 days), it appears it still was not long enough to observe increased resources to primary consumers influencing top predators as predicted in multigenerational models (Leroux and Loreau 2008). These results suggest the temporal scale of observation in experiments needs to be extended to encompass multiple generations of both primary consumer and predators, especially if effects to subsidies in slower detrital food-web compartments, such as leaf subsidies, are to be observed on top-predators. For experiments with long-lived predators such as fish, this may mean conducting experiments for several years.

Although subsidies to primary consumers did not increase top-predator biomass in my experiment, biomass of predators increased in response to the addition of insect subsidies that they directly consumed. Based on prior modelling (Leroux and Loreau 2008), I predicted that inflated predator biomass would lead to stronger top-down effects that cascaded down food webs to primary producers. However, results from Chapters Two and Three suggest that predator effects on primary consumers did not increase with increased predator biomass, likely due to the constant input of subsidies, meaning the predators were never prey-limited. These consistently strong top-down effects of fish, that did not change with subsidy input, suggest that changes in trophic casacade strength were due to interactions between primary

consumers and producers, opposed to between predators and primary consumers (DeLong et al. 2015). In my experiment, it was likely that insect subsidies, via detrital pathways, and leaf subsidies, via direct consumption, were being consumed by primary consumers in fishless tanks. Also, the strongest fish effects on primary consumers were due to increasing primary consumer biomass in the fishless tanks, as opposed to decreasing primary consumer biomass in the fish tanks. Therefore, it is most likely that, by increasing food resources to primary consumers, the interaction between primary consumers and primary producers was modified, resulting in subsidies altering trophic cascade strength. Thus, whether subsidies to predators increase trophic cascades may depend on whether these subsidies are also consumed by organisms at lower trophic levels (Dreyer et al. 2012). In particular, insect subsidies may create interactions analogous to trophic omnivory, but instead of a consumer eating across multiple trophic levels, the resources are consumed by multiple trophic levels of consumers.

RECIPROCAL SUBSIDY EXCHANGE

Few studies have investigated the role of reciprocal subsidies in highly connected ecosystems (but see Nakano and Murakami 2001, Baxter et al. 2005, Bartels et al. 2012). By manipulating the input of subsidies from the terrestrial ecosystem to multiple trophic levels of an aquatic system, I found that high-quality insect subsidies generated the largest subsequent emergence of aquatic insect back to the terrestrial ecosystem. The effect of insect subsidies on aquatic emergence was dampened by leaf subsidies, meaning that less energy was subsequently returned to the terrestrial ecosystem. Therefore, freshwater ecosystems may be sources or sinks for terrestrial resources, and the extent to which subsidies are reciprocal will depend on the type of subsidy.

Interestingly, the subsidies of emerging aquatic insects going to the terrestrial ecosystem from my tanks could play multiple roles within the terrestrial ecosystem. The majority of total emergence biomass from my tanks consisted of odonates, which as adults are predators of arthropods in terrestrial ecosystems (Corbet 1999), as well as being prey for terrestrial predators like birds (Collier et al. 2002, Murakami and Nakano 2002, Uesugi and Murakami 2007). Therefore, insect subsidies, that in my experiment, strongly increased emergence of odonates, could potentially augment predator guilds in terrestrial ecosystems, thereby generating cross-ecosystem trophic cascades (Knight et al. 2005, Schreiber and Rudolf 2008, McCoy et al. 2009). The terrestrial insect subsidies to freshwater ecosystems in my study, through increasing emerging predatory invertebrates and primary consumers, created two different potentially reciprocal connections between terrestrial and freshwater

ecosystems, either subsidising consumers such as spiders and birds (Kato et al. 2003, Akamatsu et al. 2007, Epanchin et al. 2010), or increasing predators on terrestrial consumers by bolstering predator numbers (Knight et al. 2006, Schreiber and Rudolf 2008). Therefore, it may even be inappropriate to refer to these reciprocal exchanges as cross-ecosystem subsidies since they potentially have complex roles as both resources and consumers in recipient ecosystems.

IMPLICATIONS FOR HUMAN MODIFICATION OF SUBSIDIES

Human modification of ecosystems is having landscape-wide consequences that can alter connections between ecosystems. Inputs of leaf and insect subsidies from terrestrial to freshwater ecosystems are being altered by deforestation (England and Rosemond 2004), conversion of forests to grassland and agricultural landscapes (Edwards and Huryn 1996, Kawaguchi and Nakano 2001), invasive tree species (Mineau et al. 2012), and altered fire regimes (Jackson et al. 2012). Subsidies of emerging aquatic insects to terrestrial ecosystems, in comparison, are altered by herbicides (Dewey 1986), chronic pollution (Paetzold et al. 2011), stream channelisation (Iwata et al. 2003), increased nutrients, and climate warming (Greig et al. 2012). Besides direct alteration of subsidies, alterations to the recipient trophic levels consuming subsidies can modify impacts on recipient food webs. For example, loss of or invasion of fish species can alter subsidy up-take in freshwater ecosystems, as well as insect emergence from freshwater to terrestrial ecosystems (Baxter et al. 2004, Epanchin et al. 2010, Wesner 2010).

Given the multitude of changes affecting ecosystem connections, it is important to understand how modification of those subsidies alters food-web interactions in recipient ecosystems and reciprocal flux of subsidies. My results suggest that reductions in leaf and insect subsidies to aquatic ecosystems would decrease biomass of intermediate and top predators, as well as altering primary consumer niche width and composition. This could have negative implications for ecosystem services such as fisheries, where subsidies can contribute substantially to the growth and biomass of recreational fish species (Tanentzap et al. 2014).

The importance of leaf and insect subsidies may also go beyond supporting top predator biomass. I found non-additive effects of simultaneous input of insect and leaf subsidies decreased temporal variation in primary consumer biomass and reduced trophic cascades. These non-additive effects will be important considerations for conservation efforts when restoring aquatic systems, like streams, after human modifications, such as

deforestation or agricultural conversion. Leaf and wood inputs aid stream restoration, but my results suggest that terrestrial insect subsidies may be equally important (Lake et al. 2007). In addition to the effects of reduced subsidy input on recipient food webs, human modification of subsidy flux has the potential to decrease reciprocal subsidies back to the donor ecosystem, creating a positive feedback loop that will potentially decrease connections between once highly connected ecosystems. Studying ecosystems, and the food webs within, in isolation is potentially turning a blind eye to cross-ecosystem connections that can drastically alter the function of landscapes and the ecosystem services they provide societies. My research will hopefully strongly motivate ecologists, resource managers, and conservation practitioners to consider the importance of cross-ecosystem subsidies entering recipient food webs at multiple trophic levels to the systems they are responsible for.

APPENDICES

APPENDIX 1.1

Papers used in meta-analysis, with numbering referring to Table 1.1.

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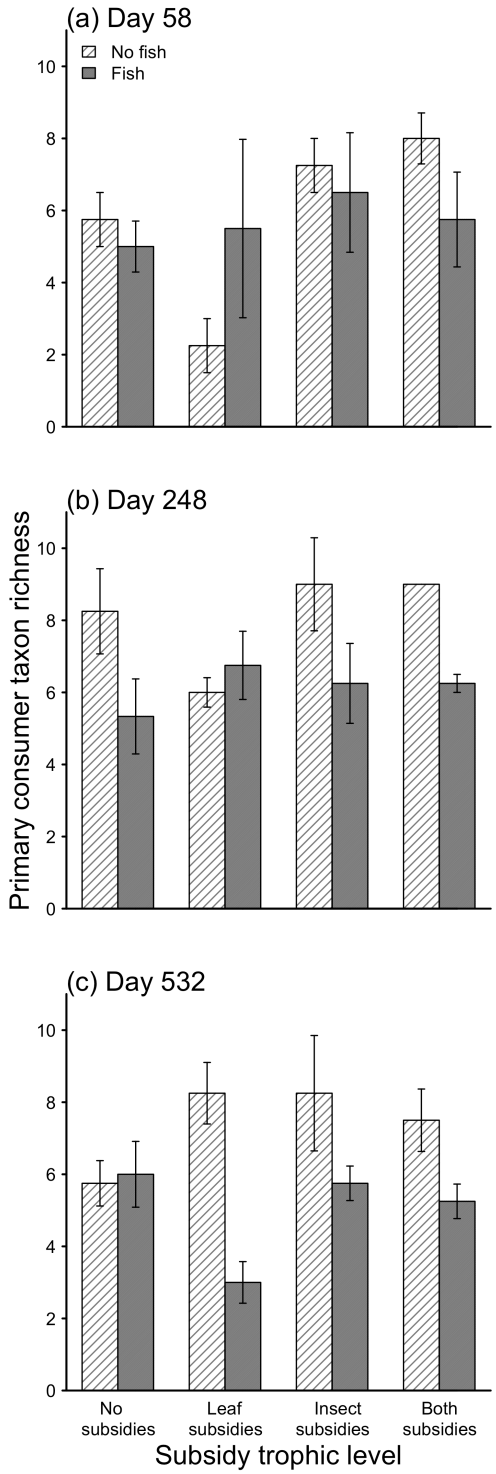
APPENDIX 3.1



Appendix 3. 1: Mean taxon richness (+/- standard error) of primary consumers for fish (solid bars) and no fish (hashed bars) treatments after (a) 58, (b) 248, and (c) 532 days without subsidies and with the addition of terrestrial leaf, insect, and both leaf and insect subsidies (subsidy trophic level).

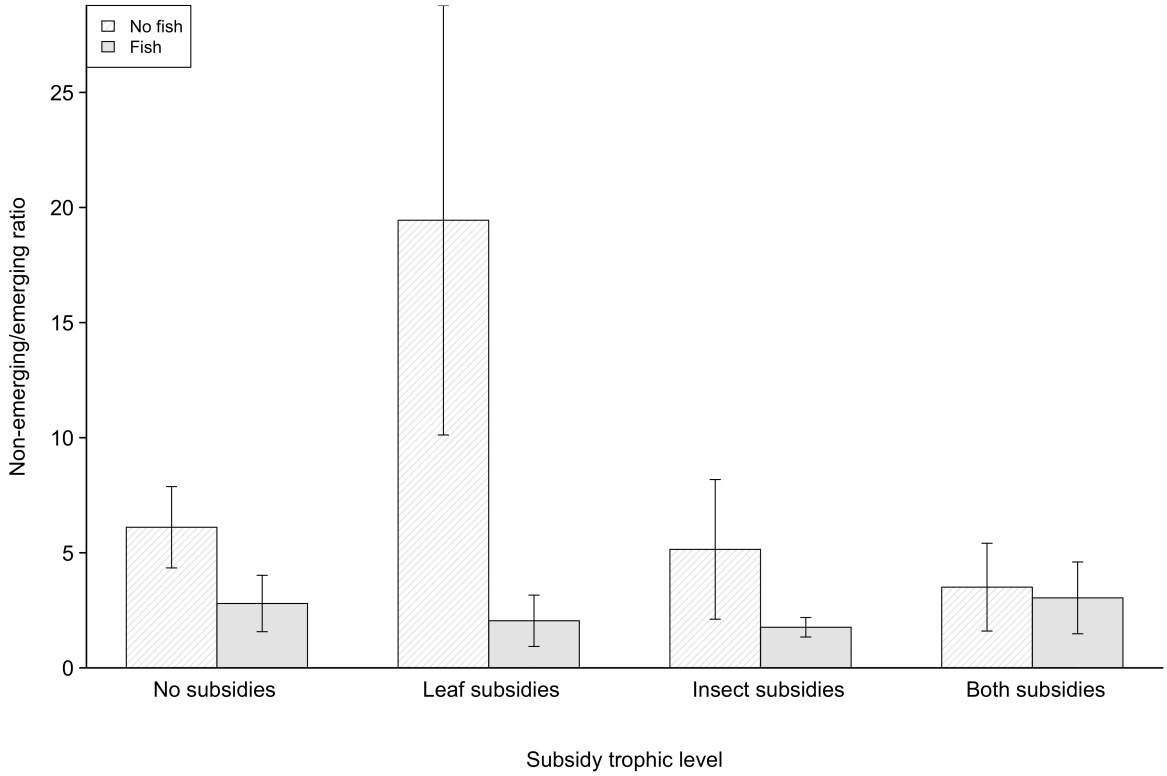
APPENDIX 4.1

Appendix 4. 1: Mixed-effects linear model for the ratio of non-emerging to emerging primary consumers on day 532 of experiment. Fixed factors are presence of insect subsidies, presence of leaf subsidies, presence of fish, and their interactions with experimental block as a random factor. Degrees of freedom were 1 and 21 for all predictor terms. Bold p-values are significant (alpha = 0.05).

Variable	F	P	analysis
Ratio non-emerging/emerging primary consumer biomass *			
			Mixed effects
insect subsidies	0.73	0.401	random = block
leaf subsidies	0.16	0.690	
fish	6.15	**0.022**	
insect x leaf	0.01	0.957	
insect x fish	1.78	0.196	
leaf x fish	0.68	0.418	
insect x leaf x fish	1.05	0.316	

* $\log_e$-transformed variable

APPENDIX 4.2



Appendix 4. 2 Mean ratio of non-emerging to emerging primary consumer biomass on day 532 of experiment with no subsidy input, terrestrial leaf subsidies, terrestrial insect subsidies, or both leaf and insect subsidies in tanks with and without predatory fish. Error bars +/- 1 standard error of the mean.

APPENDIX 4.3

Appendix 4. 3: Mixed-effects linear models for rate of emergence per-day (mg/m^2/day) on experimental day (a) 203, (b) 232, (c) 247, (d) 264, (e) 274, (f) 298, and (g) 322. Fixed factors were presence of insect subsidies, presence of leaf subsidies, presence of fish, and their interactions and experimental block as a random factors. Degrees of freedom are 1 and 21 for all predictor terms. Bold p-values are significant (alpha = 0.05).

Variable	F	P	analysis
Total biomass (mg/m^2/day)*			
a) Day 203			Mixed effects
insect subsidies	6.19	**0.021**	random = block
leaf subsidies	0.62	0.438	
fish	13.74	**0.001**	
insect x leaf	2.89	0.103	
insect x fish	2.19	0.153	
leaf x fish	0.49	0.491	
insect x leaf x fish	1.71	0.205	
b) Day 232			Mixed effects
insect subsidies	5.52	**0.029**	random = block
leaf subsidies	0.67	0.422	
fish	9.87	**0.005**	
insect x leaf	6.84	**0.016**	
insect x fish	0.82	0.374	
leaf x fish	1.67	0.209	
insect x leaf x fish	1.34	0.261	
c) Day 247			Mixed effects
insect subsidies	0.54	0.471	random = block
leaf subsidies	0.19	0.665	
fish	6.77	**0.017**	
insect x leaf	2.05	0.168	
insect x fish	0.22	0.642	
leaf x fish	1.61	0.218	
insect x leaf x fish	1.32	0.264	
d) Day 264			Mixed effects
insect subsidies	3.53	0.084	random = block
leaf subsidies	0.01	0.935	
fish	0.93	0.353	
insect x leaf	0.28	0.606	
insect x fish	0.15	0.706	
leaf x fish	0.23	0.634	
insect x leaf x fish	1.13	0.307	

* $\log_e$-transformed variable

Appendix 4.3: Continued.

Variable	F	P	analysis
Total biomass (mg/m^2/day)*			
e) Day 274			Mixed effects
insect subsidies	0.27	0.605	random = block
leaf subsidies	2.09	0.163	
fish	0.7	0.412	
insect x leaf	7.76	**0.011**	
insect x fish	11.5	**0.003**	
leaf x fish	0.26	0.615	
insect x leaf x fish	3.97	0.061	
f) Day 298			Mixed effects
insect subsidies	1.11	0.305	random = block
leaf subsidies	0.54	0.469	
fish	0.83	0.372	
insect x leaf	0.44	0.513	
insect x fish	0.74	0.398	
leaf x fish	1.26	0.274	
insect x leaf x fish	0.43	0.515	
g) Day 322			Mixed effects
insect subsidies	0.05	0.823	random = block
leaf subsidies	0.16	0.691	
fish	0.83	0.373	
insect x leaf	0.12	0.728	
insect x fish	0.24	0.623	
leaf x fish	0.01	0.894	
insect x leaf x fish	0.91	0.352	

* $\log_e$-transformed variable

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